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PATHOGENESIS OF *EUSTRONGYLIDES IGNOTUS* (NEMATODA: DIOCTOPHYMATOIDEA) IN CICONIIFORMES

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ABSTRACT: Natural (n = 157) and experimental (n = 5) infections with the nematode *Eustrongylides ignotus* are described for ciconiforms collected in Florida (USA). Larvae perforated the ventriculus in 3 to 5 hr and caused hemorrhage and bacterial peritonitis that sometimes progressed to a fibrous peritonitis with extensive adhesions. Severity of the disease was related inversely to the age of the bird and directly to the number of parasites involved. Some infections in adult birds were resolved. As a consequence of eustrongylidosis, anorexia and behavioral abnormalities resulted in emaciation and may have predisposed birds to traumatic death. Host–parasite adaptations apparently were not adequate for nestling ciconiforms as death of nestlings usually occurred before infections become patent (longer than 14 days, less than 23 days). Patent infections were found in both color morphs of the great blue heron (*Ardea herodius*), and in great egrets (*Casmerodius albus*) and snowy egrets (*Egretta thula*). We propose that birds of the family Ardeidae are the primary definitive hosts.

Key words: *Eustrongylides ignotus*, eustrongylidosis, pathogenesis, Ciconiiformes, Ardeidae, disease, Nematoda.

INTRODUCTION

Eustrongylides spp. infections have been associated with epizootics in nestling ardeids (Weise et al., 1977; Roffe, 1988; Spalding et al., 1993; C. Franson, pers. comm.) and are reported commonly in adults (Jägerskiöld, 1909; Chapin, 1926; Cram, 1933; Bowdish, 1948; Locke, 1961; Cooper et al., 1978; Windingstad and Swineford, 1981). Lesions caused by *E. ignotus*, *E. tubifex*, and *Eustrongylides* spp. have been described for many species of the family Ardeidae (Locke, 1961; Weise et al., 1977; Winterfield and Kazacos, 1977; Windingstad and Swineford, 1981; Measures, 1988a; Roffe, 1988; Spalding, 1990).

The pathogenesis of *E. tubifex*, which forms tumor-like lesions in the proventriculus of common mergansers (*Mergus merganser*), has been described by Measures (1988a) based on both natural and experimental infections. She also described experimental infections in other species, including a great blue heron (*Ardea herodius*). An experimental infection with *E. tubifex* in a single black-crowned night heron (*Nycticorax* sp.) was attempted (Cooper et al., 1978), but the duration of infection and lesions were not described.

The lesions found in ciconiforms in this study and others (Locke, 1961; Roffe, 1988) are different from those described for birds from other orders infected with *E. tubifex*. We describe both natural and experimental lesions from *E. ignotus* in ardeids and include the first descriptions of natural infections in roseate spoonbills (*Ajaia ajaja*) and white ibises (*Eudocimus albus*). Infections in these latter two species (family Threskiornithidae) are described separately, because they may not be definitive hosts.

MATERIALS AND METHODS

Natural infections

As part of an epizootiologic study (Spalding et al., 1993), 962 wading bird carcasses, predominantly nestlings, were collected from Florida (USA) during 1987 through 1991. Complete necropsies were performed on fresh and frozen carcasses, and gross lesions were described and photographed; tissues were fixed in 10% neutral-buffered formalin, and 5 μm sections were prepared and stained with hematoxylin and eosin. Parasites were collected and stored in 70% ethyl alcohol with 5% glycerine. Swabs (Culturette® collection and transport system, Marion Laboratories, Inc., Kansas City, Missouri, USA) taken from the surfaces of organs or tissues collected in sterile containers were submitted to Micrim Labs, Inc., Miami, Florida, USA, for bacterial culture. Bacteria isolated were identified using

the API-20E® system (Analytab Products, Plainview, New York, USA). Nonfermenters were identified using the BBL Minitech® system (Becton Dickinson and Co., Cockeysville, Maryland, USA). Nestlings were placed in one of five size categories based on bill length measured from the tip of the maxilla to the base of the bill on the dorsal midline. The bill length at hatching was subtracted from the smallest adult bill length, and that length was divided into five equal categories for each species, such that category I = newly hatched to category V = fledging size. Bill length growth is linear through category III in most species (Custer and Peterson, 1991), after which it slows. Thus size categories IV and V may represent longer periods. Birds in juvenal plumage collected away from the colony site (referred to hereafter as juveniles) were placed in category VI, and adults in category VII. Birds (n = 157) were placed in one of the following four categories based on interpretation of eustrongylid lesions: caused death, contributed to death, insignificant in death, resolved. Logistic analysis and analysis of variance (ANOVA) methods were used to analyze disease severity and intensity (SAS Institute Inc., 1988).

Voucher specimens from this study have been deposited within the U.S. National Parasite Collection (Beltsville, Maryland, USA, accession numbers 82333 to 82340).

Experimental infections

Six tricolored heron (*Egretta tricolor*) nestlings were collected from Frank Key, Florida Bay (25°06'N, 80°54'W) on 21 May 1990. Each was from a separate nest and was the intermediate-sized nestling of three siblings and was estimated to be between 2 and 4 days of age based on bill length. Nestlings were placed in individual containers, marked, and placed in an incubator. They were fed ad libitum four times each day with thawed fish or with fresh fish which had been examined and found to be free of eustrongylid larvae. They would take fish directly from a dish of water or, if necessary, offered with forceps. They were never force-fed. On the evening of the second day, three randomly selected birds (Nos. 2, 3, and 6) each were given four eustrongylid larvae *per os* prior to feeding. Larvae were obtained from naturally infected mosquito fish (*Gambusia holbrooki*) collected in a canal in Gainesville, Florida (Alachua County) (29°40'N, 82°20'W) and maintained in the laboratory. Larvae were extracted from fish, placed in a gelatin capsule, and fed to nestlings immediately. Control birds (Nos. 1, 4, and 5) were given empty capsules. The nest container was examined carefully during the following 2 days for evidence of regurgitated food and parasites. At each feeding, birds were examined visually and by palpation (Spalding, 1990) for evidence of parasitism. Surviving birds were humanely killed by an intracardial injection of 1 ml of Uthol® (Butler, Columbus, Ohio, USA) on Day 8 post-infection (PI) and examined at necropsy as described above for naturally infected birds.

An adult great white heron (*Ardea herodius occidentalis*) (No. 1), which had been rehabilitated but could not be released or maintained, was fed capsules containing eustrongylid larvae 14 days (one male, one female) and 3 days (two females, one male) prior to euthanasia. Feces were collected daily and examined by sedimentation technique. A complete necropsy was performed, and the location of all parasites was noted. A mature female specimen of *Eustrongylides ignotus* extracted at the time of necropsy was maintained in saline solution for 2 days, and eggs were collected. Eggs were observed for development at room temperature for 2 wk.

A second adult great white heron (No. 2) was given eustrongylid larvae in *Gambusia holbrooki* at 9, 7, 5, 3 and 2 hr prior to euthanasia. The abdomens of the fish were opened and parasites counted prior to feeding to the heron. At necropsy locations of all larvae in the heron were noted.

RESULTS

Natural infections

A total of 159 (16.5%) of 962 carcasses was infected with *Eustrongylides ignotus*, and lesions were characterized in 157 of these. Lesions found in all ardeid species examined [great blue heron, great white heron, great egret (*Casmerodius albus*), tricolored heron, snowy egret (*Egretta thula*), little blue heron (*E. caerulea*), yellow-crowned night heron (*Nyctanassa violacea*), black-crowned night heron, green-backed heron (*Butorides striatus*)] were similar and are considered collectively here. Acute lesions (32%) and chronic lesions (68%) are described separately.

Eggs of *E. ignotus* with distinct shells and presumed to be mature were found within the uterus of parasites removed from the following species: great blue heron and its white color morph, great egret, and snowy egret. Two nestlings (one great egret and one snowy egret) both 23 days of age

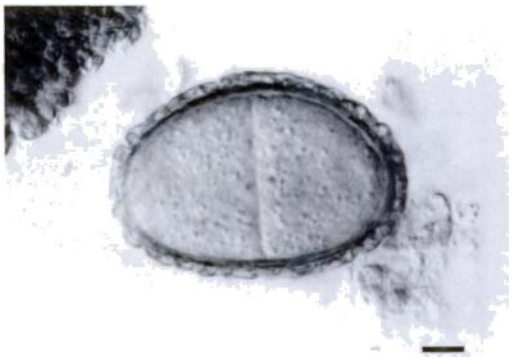

FIGURE 1. Mature egg of *Eustrongylides ignotus* from feces of a great egret demonstrating thick crenated shell and division of embryo. Bar = 10 μm.

were the youngest nestlings that contained adult female parasites with mature-appearing eggs. These eggs, when maintained in the laboratory, underwent the first embryonic cleavage, suggesting that they were fertile (Fig. 1).

Of the 24 birds containing adult female parasites with mature-appearing eggs (with a well defined shell; it is not known if all shelled eggs are fertilized), 15 (62%) of the birds were adults and post-fledging juveniles away from the colony, and 9 (38%) were nestlings still at the colony. Ten of the 24 birds also contained adult male parasites at the time of death. Only 9% of infected nestlings found dead contained female parasites bearing mature-appearing eggs, whereas 31% of infected adult and post-fledging juvenile birds contained such parasites at the time of death.

Acute lesions were those in which little evidence of inflammation was associated with the nematode, or inflammation was fibrinous in nature. Acute lesions were found in birds ranging in age from 2 days to adult. Most parasites were fourth-stage larvae (L4) (Fig. 2A), but a few were adults (none was an egg-bearing female nematode). Parasites were located most commonly below the serosa or were partly within the coelomic space on the ventricular wall (gizzard), usually on the ventral (anterior) aspect. In one extremely debilitated great blue heron, a single mature male parasite was located in the subserosa of the ventriculus, with no gross evidence of inflammation. In 15% of the cases, a subserosal hematoma measuring up to 2 cm across was associated with the parasite. In very young birds, the abdominal airsacs often contained serosanguineous fluid. The perforation site on the mucosal surface of the ventriculus usually could not be located grossly. However, one parasite was found in the act of perforating the ventriculus (Fig. 2B). Minimal hemorrhage, lysed cells, and a few granulocytic leukocytes were present. Local hemorrhage was a common feature in acute cases. The parasite often was surrounded by eosinophilic material containing bacteria and a few inflammatory cells. Lesions always were associated with the ventriculus (100%). Other organs were involved as follows; airsacs (17%), liver (15%), pericardium (13%), abdominal wall (11%), and intestine (11%). The proventriculus, kidney, cloaca, and pancreas were involved in <5% of the cases. In one case, both the anterior and posterior ends of a larva protruded through different portals into the lumen of the ventriculus. Parasites were found rarely within the lumen of the stomach and only when the stomach contained incompletely digested food.

Chronic infections were the most common observation in ardeids >1 wk of age. In most cases the parasites were adult (Fig. 3A). Firm yellow/tan, sinuous, tubular lesions, commonly intertwining, were present on the surface of the ventriculus (100%) (Fig. 4A) extending to other locations including: intestines (34%), liver (28%), airsacs (15%), gall bladder and proventriculus (10%), cloaca (9%), abdominal wall (8%), pancreas (6%), and pericardium (5%). Spleen, kidney, lung, and esophagus were involved in fewer than 5% of the cases. In severe cases, all abdominal organs were inseparable and almost unrecognizable due to the presence of large numbers of tubules. In 26% of the cases hematomas ≤3 cm in diameter were present on the surface of the ventriculus (Fig. 4B). Some of

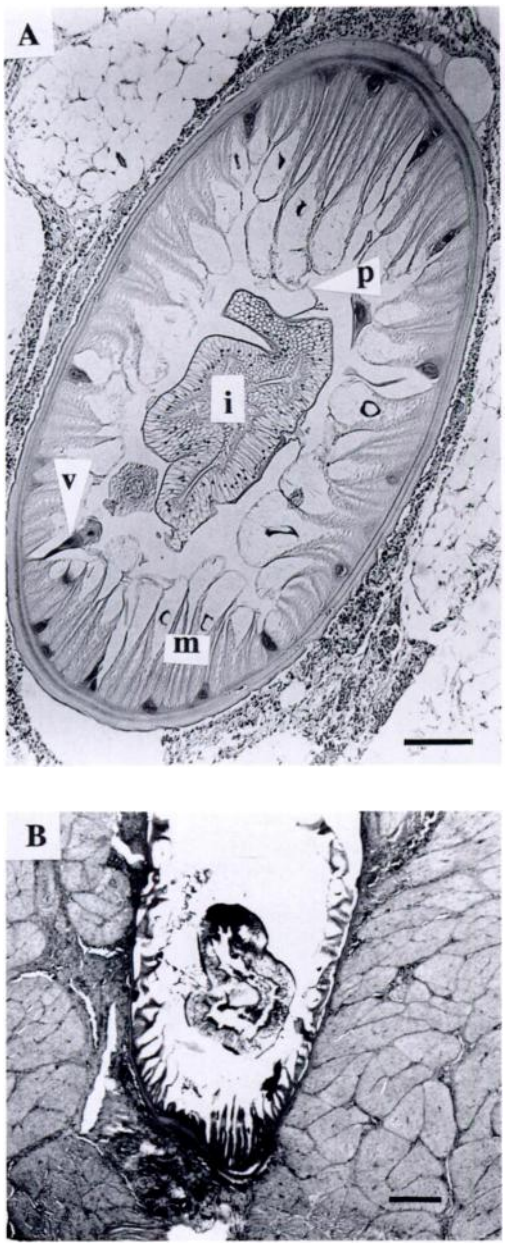

FIGURE 2. Photomicrographs of acute infections of *Eustrongylides ignotus* in a great white heron. (A) Cross section of fourth-stage larva in adipose tissue below the serosa of the ventriculus. Note the large ventral chord (v), coelomyarian musculature (m), pseudocoelomic membrane (p), and intestine (i). Bar = 100 μm. (B) Anterior end of larva penetrating tunica muscularis. Bar = 200 μm.

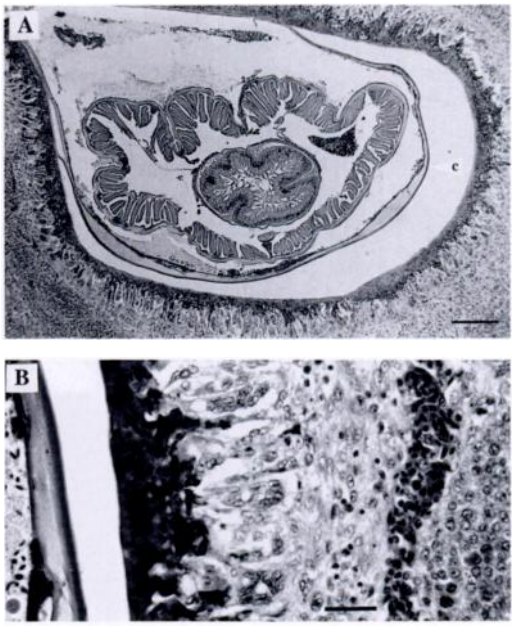

FIGURE 3. Photomicrographs of chronic *Eustrongylides ignotus* infection in a tricolored heron illustrating (A) cross section of an adult nematode with shed cuticle (c) and granulomatous reaction. Bar = 100 μm. (B) Higher magnification of granulomatous reaction with nematode cuticle to the left. Bar = 25 μm.

these had developed into abscesses containing cellular debris, bacterial colonies, and eustrongylid eggs (Fig. 5). Tubules were usually patent with the lumen of the ventriculus by way of a raised 1- to 2-mm portal (Fig. 4B). The number of portals often corresponded to the number of parasites present. The anterior extremity of the parasite, or less commonly the posterior extremity, would often protrude into the lumen of the ventriculus. Occasionally two parasites might protrude from the same portal, but two parasites never were found in the same tubule. Tubules containing large gravid females almost always had ≥1 enlarged chamber at which the parasite made a 360-degree turn and a twist. Histologically, nematodes were surrounded by a space containing loose cellular debris, bacterial colonies, fragments of cuticle, and sometimes eggs. This was surrounded by a dense mat of eosinophilic cellular debris and bacterial colonies, followed by a layer

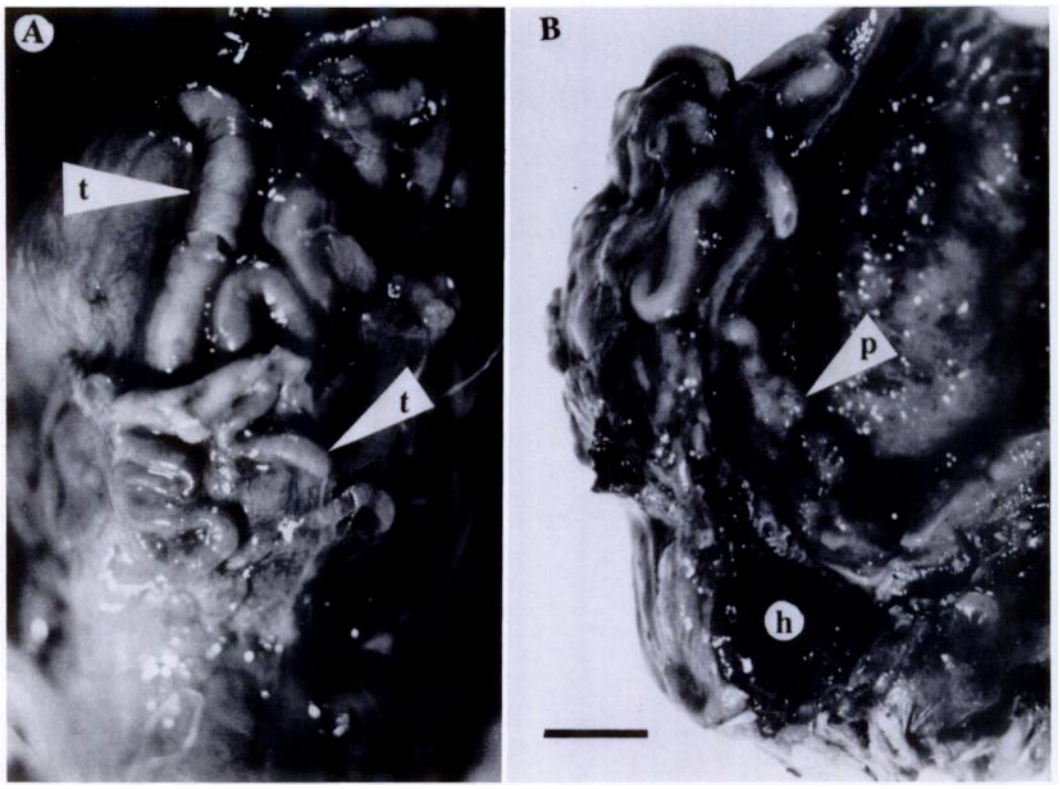

FIGURE 4. Chronic eustrongylidosis in a snowy egret. (A) Tubules (t) on surface of the ventriculus and (B) opened ventriculus illustrating a subserosal hematoma (h) and a portal (p) on the mucosal surface. Bar = 1 cm.

of multinuclear giant cells (Fig. 3B). The outermost zone was composed of fibrous connective tissue that was quite extensive in some cases. Mineral deposits in the walls of the tubules made them visible radiographically (Fig. 6). In many cases, active inflammation, characterized by fibrin, bacteria, and granulocytic leukocytes, was present also on the surface of the tubules and varied in the extent of its distribution throughout the coelomic cavity. Mild to moderate aggregations of both granulocytic (especially eosinophils) and mononuclear leukocytes were common in portal areas of the liver.

A single acute infection was found in a roseate spoonbill nestling of 129 roseate spoonbills examined. A single early fifth-stage adult male that was not yet producing sperm was coiled in the subserosa of the ventriculus near the spleen. A small amount of fibrin surrounded the parasite. Infected white ibises (5/32) were found only at one colony (26°25′N, 80°22′W) in Loxahatchee National Wildlife Refuge (Palm Beach County). Lesions in white ibis nestlings were characterized by a diffuse purulent fibrinous peritonitis, airsacculitis, and pericarditis and were less often restricted to the immediate vicinity of the parasite, as they were in ardeids. Although one immature adult female parasite was found in one white ibis nestling, no egg-producing female or mature male parasites were found in this species.

For nestlings, the probability that eustrongylidosis was the primary cause of death decreased with age (logistic regression, df = 1, $\chi^2 = 8.69$, $P = 0.0032$) (Fig. 7). Conversely, the probability that eustrongylidosis was a contributory factor in death was greater for older nestlings than

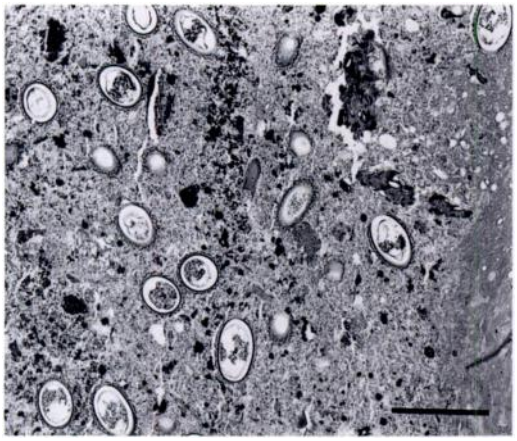

FIGURE 5. Eggs of *Eustrongylides ignotus* and cellular debris within a chronic hematoma/abscess. Bar = 100 μm.

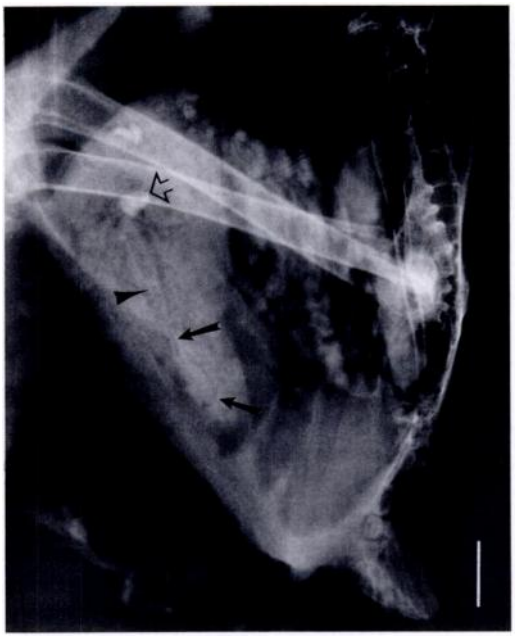

FIGURE 6. Lateral radiograph of the abdomen of a great blue heron illustrating radiodense margins of tubules and radiolucent lumens in both longitudinal (arrows) and cross section (arrowhead). Paired radiodense objects within the stomach (open arrow) are calcium storage bodies within ingested crayfish. Bar = 1 cm.

for younger nestlings (logistic regression, df = 1, $\chi^2 = 7.97$, $P = 0.0048$). In only a few cases were insignificant lesions seen in nestlings. Eustrongylidosis more commonly caused death in nestlings than in juvenile and adult birds ($n = 157$, df = 1, logistic regression, $\chi^2 = 47.71$, $P < 0.0001$). Juveniles and adults frequently had insignificant lesions. Resolved lesions, characterized by tubular scars that sometimes contained dead parasites, were found only in adult birds. Small, black, firm (sometimes tubular) granulomas were found often on the surface of the ventriculus of adult birds; likely these were resolved eustrongylid lesions.

The mean number of parasites found in 130 birds in which eustrongylidosis caused or contributed to death (range, 1 to 24 parasites) did not differ significantly among nestling size categories (ANOVA, 4 df contrast, $P = 0.4557$) (Fig. 8), nor between juveniles and adults (1 df contrast, $P = 0.4363$). The mean number of parasites for juveniles and adults differed from the mean for category I–V nestlings (1 df contrast, $P = 0.0014$).

Experimental infections

The following observations were made on tricolored heron nestlings infected experimentally in the laboratory (nestlings 2, 3, and 6 were infected with eustrongylid larvae).

In nestling 2, a dark red area was noted in the lower left quadrant of the abdomen, and a linear red parasite could be seen through the translucent abdominal skin, and felt on the surface of the ventriculus 10 hr following feeding of larvae. Five hr later a gas pocket in the coelomic cavity formed near the dark red area. On Day 2 PI a firm, dark area was palpated in the upper right quadrant of the abdomen. On Day 6 PI, four linear to serpentine, firm tracts could be felt running transversely on the surface of the ventriculus.

For nestling 3, one parasite and four fish had been regurgitated by 10 hr following feeding of larvae. At that time a serpentine parasite was both seen and palpated on the surface of the ventriculus, lower right quadrant. The nestling was weak and hypothermic. It regurgitated most of the food

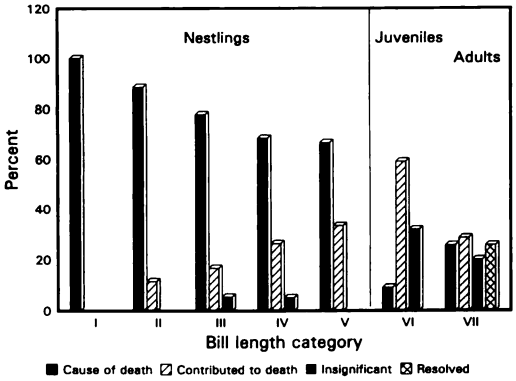

FIGURE 7. Percent of wading birds (n = 157) in each bill length category for which eustrongylidosis caused death, contributed to death, was insignificant, or was resolved.

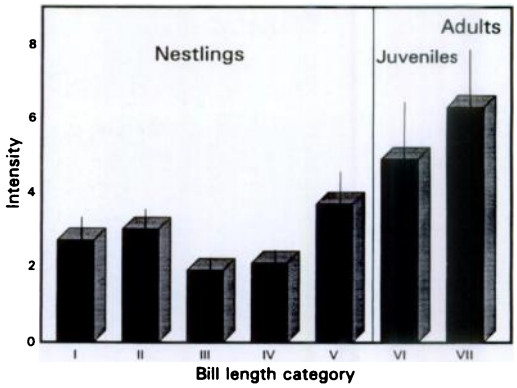

FIGURE 8. Mean intensity (±SE) of *Eustrongylides ignotus* present in 130 wading birds of various size categories for which the parasites caused or contributed to death.

it ate and would no longer eat by the evening of Day 1 PI. It died midday on Day 2 PI, 38 hr after ingestion of larvae.

For nestling 6, no evidence of parasitism was seen or palpated until Day 5 PI. At that time a hard tubular structure, 2 mm in diameter was felt in the lower right quadrant of the ventriculus.

No evidence for parasitism was felt or seen in the control nestlings (nestlings 1, 4, and 5).

Four specimens of *Eustrongylides ignotus* were found in the coelomic cavity in nestlings 2 and 6, and three were found in nestling 3. All larvae were L4 in nestling 3. Nestlings 2 and 6 had four adult parasites each; the female parasites were not egg bearing and sperm was present in the vas deferens of an adult male in nestling 6. No parasites were found in the control nestlings.

The left abdominal airsac of nestling 3 contained serosanguineous fluid. A hematoma was located in the subserosa of the ventriculus, and numerous foci of hemorrhage were found within the tunica muscularis. Aspirated ingesta probably caused suffocation. Fibrinous inflammation, characterized by large numbers of eosinophils, involved the tunica muscularis and serosa of the ventriculus, proventriculus, small intestine, mesenteries, airsacs, cloaca, and pericardium. Perivasculitis was noted near parasite tracts within the tunica muscularis of the ventriculus. The parasite was surrounded by pale eosinophilic material, erythrocytes, eosinophils and bacterial rods. There were mild eosinophilic periportal hepatitis and severe fibrinonecrotic enteritis.

Nestlings 2 and 6, which had a more prolonged exposure, are described together. Both had pyogranulomatous/fibrino-fibrous peritonitis involving the serosa of the ventriculus, proventriculus, intestines, hepatic capsule, airsacs, abdominal wall, and the pericardium. These lesions were associated with parasites and also were more generalized in the coelomic cavity involving airsacs. Parasite tracts involved the ventriculus, liver, spleen, and small intestine in both nestlings, and the pancreas, kidney, gall bladder, and cloaca in nestling 6. Tracts were similar to those described for chronic natural infections above. Bacterial contamination was marked. In addition, a severe pyogranulomatous salpingitis was noted in nestling 6, and a pericarditis in nestling 2. Adhesions observed in both nestlings were very severe.

Both nestlings 2 and 6 had marked granulocytic extramedullary hematopoiesis (EMH) in periportal areas in the liver (Fig. 9A) and in subcapsular chords in the kidney (Fig. 9C). Mitotic figures were common in these areas (Fig. 9A, inset).

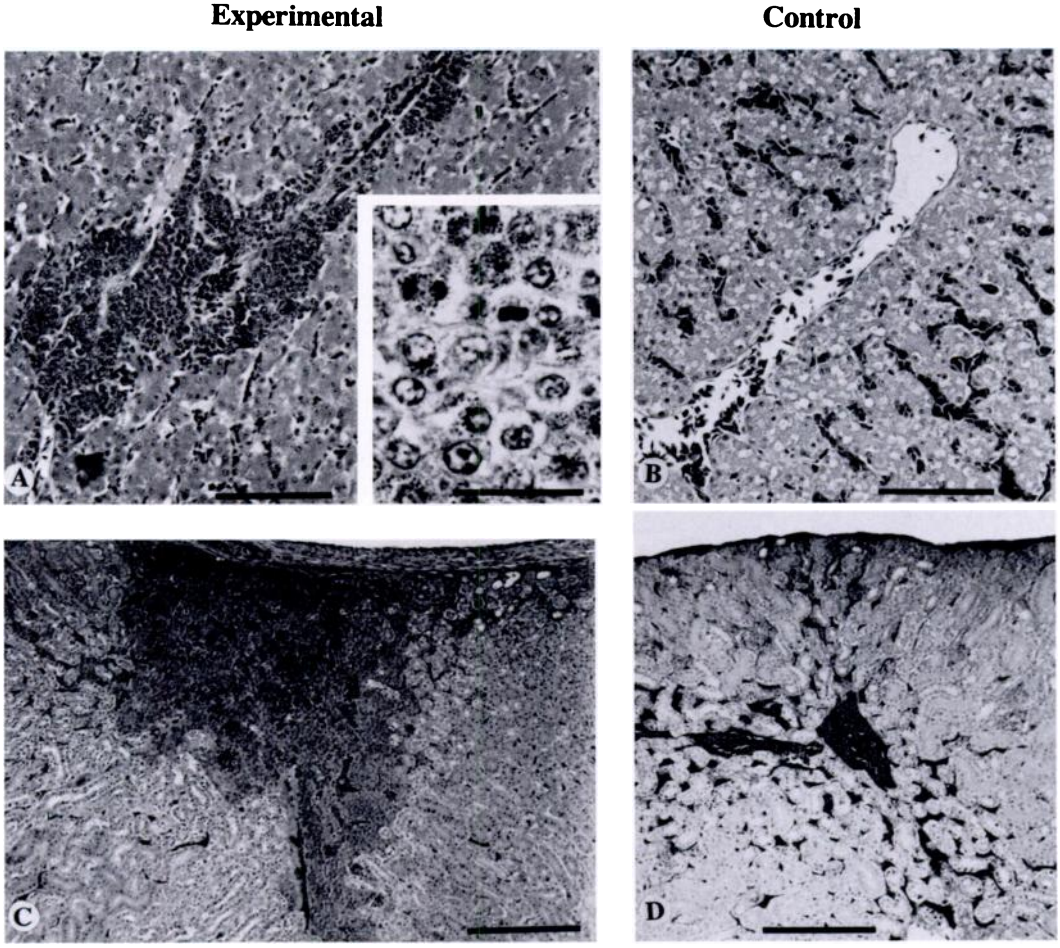

FIGURE 9. Photomicrographs of liver and kidney in experimental (A, C) and control (B, D) tricolored heron nestlings. Note marked extramedullary hematopoiesis (EMH) in portal areas of the liver (A) and in chords below the capsule of the kidney (C) in experimental infections. (A, B) Bar = 100 μm. (C, D) Bar = 200 μm. Inset is a close-up of cells in areas of EMH. Bar = 50 μm.

Control birds had no evidence of hepatitis or hepatic EMH (Fig. 9B), and only minimal evidence of renal EMH was observed (Fig. 9D). Intracellular fat vacuoles in hepatocytes were larger and more numerous than in experimental birds (Fig. 9B).

All control nestlings had abundant fat, while nestlings 2 and 6 had moderate fat, and nestling 3 had no fat.

Clostridium perfringens was isolated from all tissues examined from both experimental and control nestlings. *Alcaligenes calcoaceticus* was isolated from the liver of one infected nestling and one control nestling. *Escherichia coli* was isolated from the liver of one control nestling, and two infected nestlings. The following were isolated only from infected nestlings: *Pseudomonas aeruginosa* from the coelomic cavity, epicardium and liver of one nestling; *Aeromonas hydrophila* from the coelomic cavity of two; *Edwardsiella tarda* from the epicardium and coelomic cavity of one and from the liver of another; *Proteus mirablis* from the coelomic cavity of one and liver of two nestlings; *Plesiomonas shigelloides* from the coelomic cavity of

two nestlings; *Klebsiella pneumoniae* from the liver of one nestling; a gamma nonhemolytic *Streptococcus* group D from the epicardium of one nestling; and *Citrobacter freundii* and a heavy growth of normal gut flora from the coelomic cavity of one nestling.

At necropsy of adult great white heron 1, one adult female parasite with immature-appearing eggs in the uterus, one adult male with sperm present in the vas deferens, and two fourth-stage female larvae were found within tubular lesions. One parasite was not found. Lesions were similar to the chronic (adult) and acute (L4) lesions already described. The eggs collected from the adult female were opaque with a thin shell and showed no sign of development after 2-wk incubation. Eggs similar to immature eggs in the uterus of the female *Eustrongylides ignotus* first appeared in the feces of the bird 7 days PI.

A fish and a free larval parasite were found in the esophagus of adult great white heron 2, presumably the one given 2 hr prior to death. The lumen of the ventriculus contained two larvae and no fish; the fish given at 3 hr before death contained two larvae. Three larvae, likely those given at 5, 7, and 9 hr prior to death, were present in the tunica muscularis and in adipose tissue on the ventriculus; one of these had perforated the serosa and protruded into the coelomic cavity 3 cm. Hematomas were located multifocally in the subserosa of the ventriculus and measured ≤25 mm across.

DISCUSSION

Features of parasite maturation

Based on the location of larvae in great white heron 2, it appears that larvae perforate the stomach within 3 to 5 hr following ingestion of infected fish. This is consistent with the visual and palpable lesions noted 10 hr following infection of the captive tricolored heron nestlings. The reason for the delay in detecting a lesion in nestling 6 likely was due to the location of the perforations, which had occurred on the dorsal aspect of the ventriculus.

Molting from L4 to adult appears to take more than 2 days and less than 8, as evidenced by adult forms in nestlings killed at 8 days PI. It may take as little as 3 days, as adult parasites were found in wild 3-day-old nestlings. It appears that maturation of the female to produce mature-appearing eggs takes longer than 14 days and less than 23 days from the time of ingestion of infected fish.

Hosts

Since mature specimens of *Eustrongylides ignotus* have been described frequently in birds of the family Ardeidae, it appears that these species are common definitive hosts. In the present study patent infections occurred in three ardeid species (both white and blue color morphs of the great blue heron, great egret, and snowy egret). Observations of the severe lesions that occur in nestling ardeids, however, leads us to believe that there is little host/parasite adaptation in young birds, as nestlings often die prior to the production of eggs by the parasite.

Infections are reported here for two nonardeid ciconiforms, the roseate spoonbill and white ibis. However, mature parasites have not yet been described in these birds. Further work is necessary to define the role of these species as definitive hosts. Infections with *E. ignotus* have been reported occasionally in Pelicaniformes (Measures, 1988b). However, we could find no evidence that patent infections have been reported in these species. Eustrongylid larvae were found in white pelicans (*Pelecanus erythrorhynchos*) in Florida (Spalding, unpubl.; J. L. Burney, pers. comm.).

Host behavior

Regurgitation or vomiting as a response to ingestion of eustrongylid parasites was noticed in this study and by Measures (1988a). Hosts may be able to rid themselves of parasites in this way. Eustrongylids have no hold-fast structures, such as

are present in many lumen-dwelling parasites. Rapid perforation and residence outside of the intestinal tract may thus be a mechanism to avoid being expelled from the host.

Anorexia was noticed in experimentally infected nestlings. This, in combination with sibling competition for food, may contribute to the common finding of emaciation in naturally infected birds. Fat was much more abundant in both the surviving, experimentally infected and control birds fed ad libitum than was ever observed in wild nestlings. We suspect that the advantage of ad libitum feeding and lack of sibling competition allowed the experimentally infected birds to survive longer than they would naturally.

Infected birds may wander into unusual situations which would predispose them to mortality from other factors. In four cases, infected birds were found moribund in residential neighborhoods. A weak infected great blue heron by the side of the road was killed by an automobile the next day. Infected nestlings often were found wandering from their nests. Many of these had evidence of trauma to the head, probably as the result of attacks by siblings and other wading birds in the colony.

Pathology

Infection with *E. ignotus* results in perforation of the ventriculus and less commonly the proventriculus, attended by the formation of subserosal hematomas or hemocoelom. Many species of bacteria, probably originating from the lumen of the stomach, contaminate the coelomic cavity. Bacterial peritonitis and the formation of tubular fibrino-fibrous peritonitis, which can involve many organs in addition to the ventriculus, result from the presence of the parasites in the coelomic cavity. The parasites reside within these tubules and maintain portals to the lumen of the ventriculus. An anterior or posterior extremity may protrude into the ventricular lumen through the portal. The following consequences may contribute to or result in death: hemorrhage, bacterial peritonitis, septicemia, emaciation, organ dysfunction, intestinal obstruction, and trauma (intraspecific aggression or abnormal behavior predisposing to trauma). Control birds consumed more food per meal and grew faster (bill length and body mass) than infected birds (Spalding and Forrester, unpubl.).

Granulocytic aggregations in portal areas of the liver of the experimentally infected nestlings were difficult to interpret. Eosinophilic hepatitis in response to parasite infection and/or septicemia is one explanation, and is supported by the similar appearance of the inflammatory response at the site of the parasite in the liver. However, the uniformity of cell types, their immaturity and the presence of mitotic figures suggest an extramedullary granulocytic response. This is supported by the presence of chords of EMH in the kidneys of these same individuals. The reasons for the absence of a concurrent myelocytic response are unknown. Based on the complete lack of periportal EMH in the liver of control birds, we believe that the presence of EMH, which is thought to be a normal finding in young birds (Riddell, 1987), may in fact not be "normal," but may be a hematopoietic response to some insult that is occurring. Effects of captivity on the control birds, such as ad libitum feeding, however, should be considered also as a possible cause for the lack of EMH.

The lesions described in this study were very similar to those described in ardeids from other areas of North America (Locke, 1961; Weise et al., 1977; Windingstad and Swineford, 1981; Roffe, 1988). They differed somewhat, primarily in location of infection, from those described for *E. tubifex* (Measures, 1988a).

Eustrongylidosis was more commonly the cause of death in young nestlings than it was in older nestlings. As birds became older, and lesions more chronic, the actual cause of death became less apparent, and eustrongylidosis often appeared to contribute to, rather than cause, the death of

a bird. Rarely was eustrongylidosis considered to be insignificant in nestlings. However, insignificant lesions were found frequently in juveniles and adults. Resolved lesions were found only in adult birds.

In cases in which eustrongylidosis was a significant factor in the death of the bird, usually more parasites were found in adults than in nestlings. Thus, more parasites may be necessary to kill adults than nestlings.

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LITERATURE CITED

BOWDISH, B. S. 1948. Heron mortality caused by *Eustrongylides ignotus*. Auk 65: 602–603.

CHAPIN, E. A. 1926. *Eustrongylides ignotus* Jagersk. in the United States. The Journal of Parasitology 13: 86–87.

COOPER, C. L., J. L. CRITES, AND J. SPRINKLE FASTZKIE. 1978. Experimental and natural infections of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) in waterfowl and shore birds. Avian Diseases 22: 790–792.

CRAM, E. B. 1933. *Eustrongylides ignotus* from the black-crowned night heron. Proceedings of the Helminthological Society of Washington 20: 71.

CUSTER, T. W., AND D. W. PETERSON. 1991. Growth rates of great egret, snowy egret and black-crowned night-heron chicks. Colonial Waterbirds 14: 46–50.

JÄGERSKIÖLD, L. A. 1909. Nematodes aus Äegypten und dem Sudan. Results of the Swedish Zoological Expedition to Egypt and the White Nile 1901 3: 1–66.

LOCKE, L. N. 1961. Heron and egret losses due to verminous peritonitis. Avian Diseases 5: 135–138.

MEASURES, L. 1988a. The development and pathogenesis of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in piscivorous birds. Canadian Journal of Zoology 66: 2223–2232.

———. 1988b. Epizootiology, pathology, and description of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in fish. Canadian Journal of Zoology 66: 2212–2222.

RIDDELL, C. 1987. Avian histopathology. American Association of Avian Pathologists. Allen Press, Lawrence, Kansas, 152 pp.

ROFFE, T. J. 1988. *Eustrongylides* sp. epizootic in young common egrets (*Casmerodius albus*). Avian Diseases 32: 143–147.

SAS INSTITUTE INC. 1988. SAS/STAT® user's guide, Release 6.03 ed. SAS Institute Inc., Cary, North Carolina, 1,028 pp.

SPALDING, M. G. 1990. Antemortem diagnosis of eustrongylidosis in wading birds (Ciconiiformes). Colonial Waterbirds 13: 75–77.

———, G. T. BANCROFT, AND D. J. FORRESTER. 1993. The epizootiology of eustrongylidosis in wading birds (Ciconiiformes) in Florida. Journal of Wildlife Diseases 29: 237–249.

WEISE, J. H., W. R. DAVIDSON, AND V. F. NETTLES. 1977. Large scale mortality of nestling ardeids caused by nematode infection. Journal of Wildlife Diseases 13: 376–382.

WINDINGSTAD, R. M., AND D. M. SWINEFORD. 1981. *Eustrongylides* and pesticide levels in a great blue heron shot in Wisconsin. The Prairie Naturalist 13: 61–62.

WINTERFIELD, R. W., AND K. R. KAZACOS. 1977. Morbidity and mortality of great blue herons in Indiana caused by *Eustrongylides ignotus*. Avian Diseases 21: 448–451.

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