Journal of Wildlife Diseases, 32(3), 1996, pp. 552–555

Prevalence of *Eustrongylides ignotus* in Mosquitofish (*Gambusia holbrooki*) in Florida: Historical and Regional Comparisons

Peter C. Frederick,[1] Steven M. McGehee,[1,3] and Marilyn G. Spalding,[2] [1] Department of Wildlife Ecology and Conservation, P.O. Box 110430, University of Florida Gainesville, Florida 32611-0430 USA; [2] Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611 USA; [3] Current address: % Buck Island Ranch, 816 Buck Island Ranch Road, Lake Placid, Florida 33852 USA

ABSTRACT: We examined mosquitofish (*Gambusia holbrooki*) caught between January 1990 and December 1991 from 24 sites in northern Florida (USA), and preserved specimens collected from 32 sites throughout Florida between 1930 and 1978, for presence of the larval nematode *Eustrongylides ignotus*. We found 10 (42%) of the 24 recently-sampled sites in northern and central Florida had at least one larval nematode present in the fish; combining these data with results of previous work, 11 (15%) of 75 recently sampled sites throughout Florida had at least one nematode. By comparison, in the museum collections fish from only one (3%) of the 32 sites had the nematode, and none in northern Florida.

Key words: *Eustrongylides ignotus,* mosquitofish, *Gambusia holbrooki,* Ciconiiformes, wading birds.

The nematode *Eustrongylides ignotus* infects fish-eating ardeid birds as its final host, with oligochaetes acting as the presumed first intermediate host, and a variety of species of fish as the second intermediate host (Spalding and Forrester, 1993; Spalding et al., 1993). By burrowing through the stomach wall of the bird and into the coelomic cavity, the parasite often causes hemorrhage, bacterial infection, scar tissue formation, loss of appetite, slowed growth, and often death, especially in young birds (Spalding and Forrester, 1993). Spalding et al. (1993) found that mortality of young herons due to infection with *E. ignotus* can be as high as 80% in some breeding colonies studied. *Eustrongylides ignotus* is widely distributed in the United States, and epizootics have been described from Delaware, Louisiana, Texas, Florida, and California (Weise et al., 1977; Roffe, 1988; Spalding et al., 1993; Franson and Custer, 1994).

The distribution and prevalence of the parasite may be affected by surface water quality. Hirshfield et al. (1983) suggested that high prevalences of *E. ignotus* in the discharge of a power plant in Maryland (USA) were associated with organic enrichment, elevated year-round temperatures, and consequent high densities of oligochaetes. Several authors have noted the importance of high oligochaete densities in sites of high *Eustrongylides* spp. prevalence (von Brand, 1938; Cooper et al., 1978; Crites, 1982; Measures, 1988). Measures (1988) concluded that conditions which led to dense populations of oligochaete intermediate hosts could set the stage for epizootics of eustrongylidosis.

The distribution of *E. ignotus* in fishes in Florida has been linked with aquatic habitats having disturbed soil, exogenous nutrients, and high densities of oligochaetes (Spalding et al., 1993). Thus, pollution of surface waters may affect the distribution and prevalence of *E. ignotus* in fish in Florida.

One prediction arising from this hypothesis is that the parasite should be found more commonly in areas of denser, presumably more polluted, human habitation, and should therefore be more prevalent now than in the past. In this paper, we report on regional and historical differences in the distribution and prevalence of *E. ignotus*.

Between January 1990 and December 1991, we collected 938 mosquitofish (*Gambusia holbrooki*) by dip net at 24 sites ($\bar{X}$ = 39.1 fish/site, SE = 22.3) in north and central Florida, predominantly in Alachua County. Although *E. ignotus* may occur in a variety of fish species, we

TABLE 1. Comparison of proportion of sites with one or more fish infected with *Eustrongylides ignotus*.

	Number of sites examined	Number of fish examined	Prevalence of infected fish (%)	Mean (SD) fish examined per site	Percent of sites infected
All Florida (1990–1991)	75	3,174	1.13	42.3 (34.0)	14.7
All Florida (1930–1978)	32	724	0.14	22.6 (7.9)	3.1
North Florida (1990–1991)	24	938	2.45	39.1 (37.3)	41.7
North Florida (1930–1978)	10	242	0.00	24.2 (9.5)	0.0
South Florida (1990–1991)	45	1,686	0.77	37.5 (30.9)	2.2
South Florida (1930–1978)	22	482	0.23	21.9 (7.3)	4.5

report only prevalences found in mosquitofish in an effort to standardize our comparisons. This species is ubiquitous in Florida, known from past work to frequently harbor larvae of *Eustrongylides* spp., easy to capture in large numbers with a dip-net, and broadly represented geographically in museum collections. Fishes were killed by freezing, and the entire body dissected to search for larvae of *Eustrongylides ignotus*. Dissected fishes were examined for larvae with a binocular dissecting microscope (10 to 30×). Although eustrongylid larvae cannot be identified to species, we presumed that all were *E. ignotus* based on the results of experimental infections by Spalding and Forrester (1993). Samples were taken from all major wetlands and streams in Alachua County, and we included a variety of both pristine and human-altered sites. Voucher specimens of larval estronglids deposited with the U.S. National Parasite Collection (Beltsville, Maryland, USA) are USNPC 84898-00 and 84900-0, in collection SH 117–29.

We also dissected 724 specimens of *G. holbrooki* preserved in alcohol at the Florida Museum of Natural History in Gainesville, Florida. These fishes were collected from 32 sites throughout peninsular Florida between 1930 and 1978, including 10 from Alachua County. Samples were rejected if the fish had any signs of decomposition or drying. Up to 30 fish were randomly selected and dissected from each sample. Mean ± SE numbers of fishes examined per collection site was 22.6 ± 7.74. Ranges of latitude and longitude in both recent and historical samplings was 25°30′N, 80°22.5′W, to 29°00′N, 83°00′W. Fisher's exact test (Siegel, 1956) was used to test for differences in prevalences of *Eustrongylides* spp. in the recent and historical samples.

Between 1990 and 1991 in Alachua County we found 10 (42%) of the 24 sites sampled had at least one fish carrying at least one eustrongylid larva (Table 1). We found 23 (2.5%) of 938 fish infected.

In the 724 museum samples from all of Florida, we found only one (0.1%) fish infected; this was at one (3%) of the 32 historical sites in Florida, found in Collier County in 1952. Within Alachua County, the dates of museum samples ranged from 1930 through 1963, and none of the 10 samples (242 fishes) contained *E. ignotus*. In Alachua County, this prevalence among museum collections was significantly (P = 0.015) lower than in recent samples.

Combining our findings with the data from Spalding et al. (1993) from south and central Florida, collected between 1989 and 1990, the total for peninsular Florida adds up to 11 (15%) infected sites found in 75 recently sampled sites. This prevalence in recently-sampled sites is significantly ($P < 0.05$) greater than that measured from the historical samples throughout the state.

From these comparisons, we propose that historical prevalences of the parasite are much lower than those since 1989. It is unlikely that this latter result was an effect of a differential detectability of *E. ig-*

notus in preserved versus frozen specimens. Although eustrongylid larvae are more apparent in frozen tissue because of the red color, we encountered no difficulty in locating parasites in formalin or alcohol-fixed fish from recent collections. Because of the relatively large numbers of sites involved in this comparison, we believe that the differences found were not an artifact of inadequate sampling effort.

We also propose that the prevalence of *E. ignotus* in *G. holbrooki* is currently much higher at sites in north Florida than at southern Florida sites (42% versus 5%, $P < 0.001$). Differences between regions could be due to differential habitat suitability. In subsequent analysis of the southern Florida data, we found no *E. ignotus* at sites with highly organic substrates. Highly organic peats are typical of much of the southern Florida landscape, whereas soils in northern Florida are typically dominated by quartz sands (Brown et al., 1990). Explanations for this association between parasite and soil type are lacking. Wading bird numbers have declined throughout the state (Runde et al., 1991) and thus cannot account for either the regional or historical differences in prevalence.

We believe that differences in prevalences of *E. ignotus* between museum and recent collections are most likely explained by recent changes in habitat conditions, since the comparisons were of the same water bodies and in many cases, the same sites. The higher prevalences of nematodes in fishes during the present period could be explained by changes in water and soil quality during recent history, because of the influence of these changes on populations of the oligochaete intermediate host (Hirschfield et al., 1983).

Although we did not measure water or soil quality parameters at the sites we sampled, we noted that the sites in which we found *E. ignotus* in all cases also had some obvious source of exogenous nutrients, such as effluent from residential or urban septic systems, farm runoff, leaking municipal sewage, and stormwater from urban lawn runoff. We found no parasites at many other sites where a similar source of nutrients was apparent. This pattern then mimics that found by Spalding et al. (1993), and is evidence that there are other, unknown factors such as visitation by wading birds, that are important in the distribution and prevalence of the parasite.

The strong relationship between nematode occurrence and eutrophic condition may help explain the difference between historical and recent prevalences in Florida. Many Florida aquatic habitats are naturally oligotrophic (Brenner et al., 1990), and are likely to have been so during the collection of many of the museum specimens. Florida has experienced extremely rapid growth in human population and drastic changes in land use during the past 20 yr (Bouvier and Weller, 1992), which has caused many water bodies to become eutrophic. Wading bird behavior may help to form a positive feedback loop in nematode prevalence under these conditions, because ciconiiform birds appear to be strongly attracted to the high secondary productivity typical of eutrophic and hypereutrophic sites in Florida (Edelson and Collopy, 1990; Hoyer and Canfield, 1990; Frederick and McGehee, 1994). At present, increasing nutrient inputs into Florida waters is the most parsimonious explanation for the increase in prevalence of *Eustrongylides ignotus* through time.

We thank D. J. Forrester, D. Coyner and four anonymous reviewers for help in improving earlier drafts of this paper. We are indebted to C. Gilbert of the Florida Museum of Natural History for allowing use of the mosquitofish collections for our study. This work was supported by grants from the Florida Game and Freshwater Fish Commission and the Environmental Protection Agency. This is Journal Series R-04896 of the Florida Agricultural Experiment Station.

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Received for publication 24 July 1995.