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Trichobilharzia physellae (DIGENEA: SCHISTOSOMATIDAE) FROM ENDEMIC WATERFOWL ON THE HIGH PLAINS OF TEXAS

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Abstract: Adult *Trichobilharzia physellae* were recovered from the liver and mesenteric veins of green-winged teal, *Anas creca*, blue-winged teal, *A. discors*, and pintail, *A. acuta*, breeding on the High Plains of Texas. Wild ducks naturally-infected with these schistosomes were emaciated with mild to severe atrophy of the breast musculature. The liver was considerably enlarged, pale and somewhat friable. Numerous viable and dead *T. physellae* were recovered from the cut surface. On histologic examination, viable adult schistosomes were observed in the portal veins and dead, partly calcified *T. physellae* were found in the interlobular bile ducts. The normal liver architecture was well preserved but there was mild to extensive fibroplasia of most portal triads. An intense inflammatory response consisting mainly of eosinophils and fibroblasts with a few histiocytes, epithelioid cells, plasma cells, and lymphocytes surrounded the infected triads. There was pressure atrophy of adjacent hepatic parenchyma, hyperplasia or atrophy of the bile duct epithelium, bile stasis in smaller interlobular ducts, ectasia of larger portal ducts, focal areas of inflammatory cells throughout the liver, and periphlebitis of portal veins. The basic lesion presented as an obstructive fibrosis of the portal triads. These lesions are compared with those reported in infections by other schistosome species in their respective hosts. The oculate, apharyngeate, furcocercous cercariae of *T. physellae* were recovered from the snail first intermediate host, *Physa anatina*. Attempts to infect domestic mallard ducks via skin penetration by these cercariae were unsuccessful. This is the first record of a cercarial dermatitis-producing schistosome species from the Texas Panhandle and the first detailed description of such extensive lesions produced by adult schistosomes in the liver of a vertebrate host.

INTRODUCTION

The semiarid Texas Panhandle contains over 19,000 shallow, ephemeral bodies of water known regionally as playa lakes.[7] Most of these are supplemented by irrigation runoff and are full during the spring and summer months. These lakes are used for breeding purposes by at least six species of endemic waterfowl.[6] Necropsy of several specimens of immature and adult ducks at the beginning of a botulism epizootic in late summer revealed pale, enlarged livers containing large numbers of trematodes subsequently identified as *Trichobilharzia physellae*. This paper describes the liver lesions caused by adults of *T. physellae* in waterfowl from the Texas Panhandle, substantiates previous findings on the behavior and morphology of the cercariae of *T. physellae* and its snail host, and discusses selected aspects of the comparative pathology of this and other species of avian schistosomes in their respective hosts.

MATERIALS AND METHODS

Sick or disabled ducks collected in July, August and September, 1977, from Castro County, Texas, were transported

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to the laboratory and subsequently examined. Trematodes recovered at necropsy were fixed in acetic acid-formaldehyde-ethyl alcohol (AFA), stained in Semicohn's acetic carmine and mounted in Canada balsam. Sections of liver, lung, intestine, mesenteric veins, heart, kidney, spleen, and pancreas were fixed in buffered 10% formalin. Tissues were sectioned at 4 to 6 μm sections and were stained with hematoxylin and eosin, Giemsa and/or Masson's trichrome stains. Sera from some specimens were forwarded to the U.S. Fish and Wildlife Service Laboratory, Madison, Wisconsin, for testing against endotoxin of type C *Clostridium botulinum* using the mouse protection test.

Approximately 5,000 snails, identified as *Physa anatina,* were collected from selected playa lakes with breeding waterfowl populations, transported to the laboratory and examined daily for shedding cercariae. Living cercariae stained with Nile blue sulfate and/or neutral red and specimens fixed in hot AFA stained with Semicohn's acetic carmine were examined microscopically. The latter preparations were used for measurements.

Four domestic mallard ducks approximately 6 weeks old were restrained and their feet exposed for 1 h to 50, 100, 500, or 1,000 cercariae. Two ducks were maintained as controls. Three weeks after exposure these birds were examined for schistosomes.

RESULTS

All wild ducks examined, including one immature (approximately 1 month old) and one adult blue-winged teal *(Anas discors),* one adult pintail *(A. acuta),* and two adult green-winged teal *(A. creca),* were infected with *T. physellae.* All these birds demonstrated varying degrees of clinical manifestations of acute botulism including partial to complete paralysis of the cervical, wing and leg muscles, foul smelling diarrhea and soiled vent, and half-closed pasted eyelids. Also, in these hosts there was evidence of a more chronic disease process including emaciation with atrophy of the breast musculature and otherwise poor condition, but there was no evidence of ingestion of lead shot. The pintail and one green-winged teal were confirmed to be infected with *Clostridium botulinum* type C using the mouse protection test.

On necropsy the liver of ducks infected with *T. physellae* was grossly enlarged and pale. In some cases, there was a small quantity of pink fluid in the abdominal cavity. On cut section the liver was friable and contained large numbers of viable *T. physellae* in the portal veins and dead, degenerating schistosomes in the interlobular bile ducts. Predominantly male schistosomes were removed from the veins, while only females were recovered from bile ducts.

Histologic examination revealed that the normal liver architecture was well preserved, but there was mild to extensive fibroplasia of the portal triads with extension into the adjacent parenchyma. In most triads viable adult schistosomes were numerous in the portal veins and dead, partially calcified *T. physellae* were present in the interlobular ducts (Fig. 1). Portal areas had an extensive infiltration of eosinophils and fibroblasts with fewer histiocytes, epithelioid cells, plasma cells, and lymphocytes (Fig. 2). Changes in infected portal bile ducts varied from hyperplasia to complete disquamation of the duct epithelium depending on the number of schistosomes present. There was bile stasis in some smaller portal bile ducts, some of which were completely occluded. There was mild to severe medial hyperplasia of infected portal veins with periphlebitis extending into the tunica adventitia (Fig. 2). Similar changes were noted in hepatic arteries in severely affected triads, but schistosomes were not observed in the arterial circulation. There was atrophy and necrosis of

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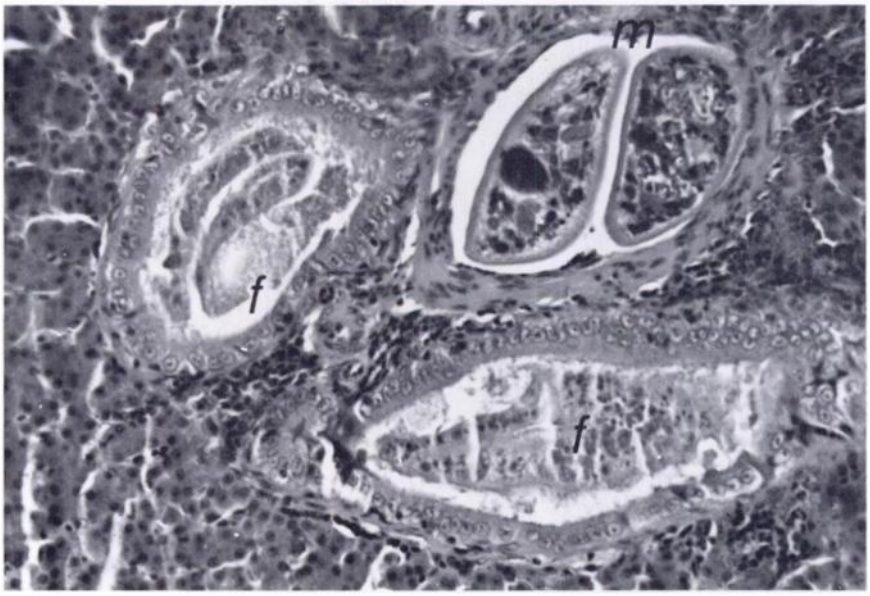

FIGURE 1. A portal triad with viable male (m) *Trichobilharzia physellae* in the portal vein and numerous degenerating female (f) schistosomes in adjacent bile ducts. Note occlusion and hyperplasia of bile ducts. H & E.

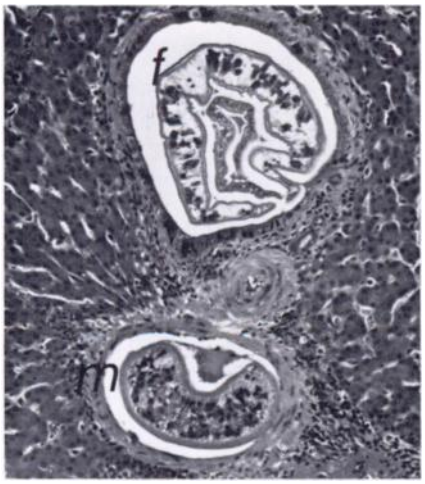

FIGURE 2. A portal triad with viable male (m) and dead female (f) *T. physellae* in the portal vein and bile duct, respectively. Note the surrounding inflammatory response. H & E.

hepatic parenchymal cells adjacent to infected triads with extensive portal fibroplasia. Hepatic cell nuclei in these areas were karyolytic. Eosinophils were scattered throughout the parenchyma in affected lobules. Also, numerous macrophages laden with hemosiderin were observed in the hepatic parenchyma. The basic lesion of the *T. physellae* infection in these hosts presented as an obstructive fibroplasia and accompanying chronic inflammatory response in the portal triads and adjacent periportal spaces and necrosis of adjacent hepatic parenchyma. This is attributed, in large part, to the aberrant localization of female schistosomes in the bile ducts. In triads containing only male schistosomes in the portal veins, little or no response was noted. No schistosomes were recovered, nor were lesions noted, in extra-hepatic bile ducts or gall bladders. Eggs were not observed in liver sections.

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Although mature schistosomes were observed in mesenteric veins, there was no evidence of a tissue response in this site. Small focal granulomas were occasionally observed surrounding schistosome eggs in the mucosa and submucosa of the intestine. Schistosomes were not observed in other tissues or organs examined microscopically.

The shallow mud flats of playa lakes in late summer are very favorable habitats for large populations of snails. *Physa anatina* is the overwhelmingly predominant species. Approximately 5,000 *P. anatina* were collected from these playa lakes. Large numbers of oculate, apharyngeate, furcocercous cercariae were shed from these laboratory-maintained *P. anatina*. Of 200 snails examined, approximately 1 in 5 dissected *P. anatina* were infected with sporocysts typical of those described for the genus *Trichobilharzia*.[9] On the basis of meristic characters, these cercariae could not be differentiated from either *T. stagnicolae* or *T. physellae* (Table 1). However, morphologically the position of the ventral sucker was more anteriorly located than described in *T. stagnicolae* and the behavioral characteristics closely resembled *T. physellae*.[1] These cercariae were vigorous swimmers, did not remain suspended in water, and often attached to the bottom of the container by means of the ventral sucker.

Gross and microscopic examination of tissue sections of the liver, mesenteries, and intestine of the four experimentally-infected ducks revealed no evidence of *T. physellae* infections.

DISCUSSION

Trichobilharzia physellae was described from cercariae obtained in naturally infected snails, *Physa parkeri* and *P. magnalacustris*, from the Douglas Lake region, Michigan.[1] The adults were described from specimens in pigeons, wild mallard ducks, and canaries experimentally-infected with cercariae from *P. parkeri*.[4] Adult schistosomes recovered from waterfowl and cercariae shed from *Physa anatina* in the Texas Panhandle correspond in all respects to the original description of the species.

This schistosome has been implicated as a causative agent in outbreaks of "swimmer's itch" or cercarial dermatitis in the Great Lakes region.[8] Although no human cases have been reported in the Texas Panhandle, the substantial number of breeding ducks, high populations of *P. anatina*, and apparent high levels of infection in intermediate (snail) and definitive (duck) hosts in-

TABLE 1. Comparison of meristic characteristics of avian schistosome cercariae. Measurements in microns.

Characters	*Present Study	**T. stagnicolae	**T. physellae
Length of body	†244 ± 15	260 ± 13	265 ± 8
Width of body	65 ± 4	60 ± 7	60 ± 6
Diameter of sucker	18 ± 3	30 ± 3	29 ± 2
Distance from ventral sucker to posterior of body	68 ± 6	60 ± 5	80 ± 5
Length of tail	301 ± 7	400 ± 7	374 ± 11
Width of tail	36 ± 4	40 ± 4	40 ± 4
Length of tail furca	157 ± 4	218 ± 19	196 ± 8
Width of tail furca	18 ± 1	25 ± 3	32 ± 1

*Based on 10 specimens
**Modified from Cort and Talbot (1936)
†Mean values followed by standard error

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dicate an excellent potential source for outbreaks of cercarial dermatitis. This is the first report of a dermatitis-producing avian schistosome from endemic waterfowl populations on the High Plains of Texas.

The pathology of avian schistosomiasis varies with different schistosome species, with various hosts, and with the same species in different hosts. The lesions of schistosomiasis are ussually egg-induced rather than a result of adult or immature schistosomes. While the following examples cite immature or adult schistosome-induced lesions, these are usually incompletely described. Growth in ducks, gulls, and canaries was retarded and liver and lung pathology due to migrating schistosomes were mentioned, but not elucidated, in experimental infections of *Bilharziella yokogawi*.[4] An enlarged abscessed liver resulting from migrating immature schistosomes was reported in a pigeon exposed to *Cercariae elvae*.[3] Mallard ducklings experimentally-infected with *Trichobilharzia ocellata* were reported to have liver damage and immature and adult schistosomes were recovered from the liver, but the lesions were not discussed.[4] Only male schistosomes were removed from the liver and various branches of the intrahepatic portal veins. Female schistosomes were not recovered. Mallard ducks showed little evidence of lesions, but male and female schistosomes were recovered from the lung, liver, and intestine.

The present study emphasizes the extent of lesions due to adult *T. physellae* in certain duck species. Similar to previous studies,[4] a predominance of male *T. physellae* were found in the portal veins, while dead and degenerating females were observed within the bile ducts. The mechanism for localizing in the bile ducts remains to be elucidated. Possibly, they migrate across the endothelial lining of the portal vein through the bile duct epithelium following the lung-liver migration. Perhaps this results from massive infections in waterfowl other than the species of duck which serves as the normal definitive host. Also, the influence of a concurrent infection such as botulism on the schistosome infection is unknown. While the aberrant localization of female schistosomes could be a response to *C. botulinum* toxin, the lesions induced by *T. physellae* are indicative of a chronic infection. Ducks with acute botulism usually succumb rapidly. Hence, the *C. botulinum* infection is regarded herein as unrelated to the schistosome infection. The lesions of *T. physellae* in the liver of ducks from the Texas Panhandle are similar to those observed in the bile ducts of ducks and swans from Michigan[2] and cormorants from Louisiana[5] infected with adults of the opisthorchiid trematode, *Amphimerus elongatus*. These differ from the typical granulomas in livers caused by the eggs as reported in many schistosome infections. Although a few granulomatous lesions surrounding eggs were noted in intestines of infected ducks examined in this study, eggs were infrequently observed and few adult schistosomes were recovered from the mesenteric veins.

Although experimental infections were attempted with *T. physellae*, domestic mallard ducks were not infected after exposure to large numbers of cercariae. Experimental infections of *T. physellae* have been established in pigeons, wild mallard ducks, and canaries, but the same authors failed to infect terns, chickens, gulls and domestic ducklings.[4]

While this study documents the presence of chronic liver disease attributable to *T. physellae* in waterfowl, a number of aspects of this infection remain to be elucidated. These include the behavioral differences in male and female schistosomes and the exact pathogenesis of the lesions. Although the aberrant localization of females in the bile ducts appeared to, in large part, account for the lesions noted in this

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infection, this needs clarification. Also, the effect of concurrent botulism on the pathogenesis of *T. physellae* is unclear. Finally, further studies on the prevalence of *T. physellae* in various waterfowl species and establishment of experimental infections in a variety of species are warranted in order to determine the normal and aberrant hosts and pathogenicity of this schistosome.

Acknowledgements

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