

Field and Laboratory Study Evaluating the Possibility of *Manodistomum syntomentera* Causing Malformations In Frogs of the Mississippi River Valley

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ABSTRACT

The past few years, the increased prevalence of deformed frogs in the Wisconsin/Minnesota area has caught the attention of scientists and environmentalists around the country. The health of frogs, and other amphibians, in an environment can be used as an indicator of the health of that environment. The objective of the project was to find out if the Mississippi River Valley harbored deformed frogs, and if the number of cases identified was unusually high. The project also looked to find out if *Manodistomum syntomentera* might be one causative agent for frog deformities. Frog collections were made at Goose Island County Park, (La Crosse Co), Van Loon Wildlife Area, (La Crosse Co), and Trempealeau National Wildlife Refuge, (Trempealeau Co). No deformities were identified in any of 12 green (*Rana clamitans*) or 35 leopard (*R. pipiens*) frogs from Goose Island, eight green frogs and two leopard frogs from Van Loon and 44 leopard frogs and 58 green frogs from Trempealeau. Three of 58 (5.17%) green frogs from Trempealeau were deformed. Deformities included missing digits, extra digits, missing extremities, and misshapen leg bones. No supernumerary limbed frogs were found. We necropsied 76 frogs, (45 *R. pipiens* and 31 *R. clamitans*) and obtained numerous parasites from the frogs, including: *Halipegus* (Eustachian tubes), *Haematoloechus* (rectum), *Rhabdias* (lungs), *Ozwardocruzia* (intestine), *Glythelmins* (intestine), *Megalodiscus* (rectum), opalinids (rectum), and gorgoderids (urinary bladder). Blood smears have yet to be stained. Metacercariae recovered include: *Manodistomum* (subdermal), neascus (subdermal) diplostomula (muscles), heterophyid (unencysted along femurs), and echinostome (kidney). These data indicate that *M. syntomentera* is not likely the causative agent of deformities in *R. pipiens* and *R. clamitans*.

INTRODUCTION

Manodistomum syntomentera is a garter snake (*Thamnophis* spp) parasite that has been incriminated as the cause of massive deformities in frogs throughout the continental United States (Sessions and Ruth, 1990). So far misshapen frogs have been found in Missouri, Minnesota, Wisconsin, Iowa, South Dakota, Ohio, Michigan, Alabama, California, and the Canadian province of Quebec. Frogs may have multiple legs (up to twelve legs per frog), stump legs, or missing eyes. At a hastily called conference in 1996, researchers speculated that the causes for frog deformities might include water pollution, UV radiation, ozone depletion, and/or parasites. In an attempt to stimulate the insult that parasite host stages might have on the limb buds of developing tadpoles, Sessions and

Ruth implanted small plastic beads into the region where hind limbs develop in tadpoles, and were able to generate similar malformations (Sessions and Ruth, 1990).

MATERIALS AND METHODS

Frogs were collected by using dip nets during the day at Goose Island County Park (La Crosse Co., WI), Van Loon Wildlife Area (La Crosse Co., WI), and Trempealeau National Wildlife Refuge (Trempealeau Co., WI), (Vogt, 1981). Frogs from each site were examined for skeletal deformities. Representative subsamples of frogs from each site were necropsied for parasites (skin, lungs, blood, GI tract, urinary tract, Eustachian tubes, peritoneal cavity, eyes, and muscles). Particular attention was paid to finding metacercarial stages of various digenetic trematodes encysted in musculature. Some tadpoles were collected at various sites and also examined for trematodes and metacercariae. Snails were collected at each of the sites where frogs were collected and screened for shedders. Snakes were collected where possible and examined for *Manodistomum*. All parasites were identified using Ulmer, M. J. (1970), Schell (1970), and Yorke and Maplestone (1969).

RESULTS

Parasites recovered in the present study are summarized in Table 1. and Plate 2. No deformities were identified in any of 12 green (*Rana clamitans*) or 35 leopard (*R. pipiens*) frogs from Goose Island, eight green frogs and two leopard frogs from Van Loon and 44 leopard frogs from Trempealeau. Three of 58 (5.17%) green frogs from Trempealeau were deformed. Deformities included missing digits, extra digits, missing extremities, and misshapen leg bones. No supernumerary limbed frogs were found. We necropsied 76 frogs, (45 *R. pipiens* and 31 *R. clamitans*) and obtained numerous parasites from the frogs, including: *Halipegus* (Eustachian tubes), *Haematoloechus* (rectum), *Rhabdias* (lungs), *Ozwardocruzia* (intestine), *Glythelmins* (intestine), *Megalodiscus* (rectum), opalinids (rectum), and gorgoderids (urinary bladder). Blood smears have yet to be stained. Metacercariae recovered include: *Manodistomum* (subdermal), neascus (subdermal), diplostomula (muscles), heterophyid (unencysted along femurs), and echinostome (kidney).

DISCUSSION

Out of the 159 frogs collected in the Mississippi River Valley only three had deformities. All three specimens had deformities of the legs; all three deformed frogs were kept by personnel of the U. S. Fish and Wildlife Service and were not available for examination of parasites.

Seventy-six of the 159 frogs that were examined for external deformities were also necropsied and examined for internal parasites. Particular attention was paid to identifying metacercariae of the musculature since *Manodistomum* metacercariae were hypothesized to be one cause for development of frog deformities in California (Sessions and Ruth, 1990). A total of five species of digenetic trematode metacercariae were found in the 76 necropsied frogs of the Mississippi River Valley (Plate 2). The metacercariae were found in the musculature of the front and rear legs and the abdomen. Musculature infections ranged from mild (20-50) to severe (100-500). Although these frogs had

infections with metacercariae, none had deformities.

In spite of often heavy metacercarial infections, Mississippi River Valley frogs have remarkably few malformations. Many other parasites were found in the frogs from the Mississippi River Valley including parasites in the lungs, GI tract, and Eustachian tubes. Deformation rates of frogs in the Mississippi River Valley near La Crosse are not nearly as high as reported for other sites within WI. Subsequent laboratory studies will be conducted in which tadpoles will be exposed to different intensities of cercariae of various digene species in an attempt to elicit deformities.

CONCLUSION

Frogs from WI are heavily infected with a number of worm parasites in the lungs, GI tract, and Eustachian tubes. Five species of digenetic trematode metacercariae occur in frogs from the Mississippi River Valley. Deformation rates of frogs in the Mississippi River Valley near La Crosse are not nearly as high as reported for other sites within WI. High rates of infection with metacercariae observed in frogs from the current study would indicate that parasites play no role in formation of abnormalities. Subsequent laboratory studies will be conducted where by tadpoles will be exposed to different intensities of cercariae of various digene species in an attempt to elicit deformities.

LITERATURE CITED

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TABLE 1. PREVALENCE AND MEAN INTENSITY OF METACERCARIAL-INFECTION IN VARIOUS MISSISSIPPI RIVER VALLEY AREAS.

Area	SPECIES	# OF FROGS DISSECTED	MEAN	PREVALENCE
GOOSE ISLAND	<i>Rana pipiens</i>	11	553	55%
	<i>Rana clamitans</i>	3	177	67%
VAN LOON	<i>Rana pipiens</i>	9	15	100%
	<i>Rana clamitans</i>	8	195	88%
TREMPEALEAU	<i>Rana pipiens</i>	25	27	100%
	<i>Rana clamitans</i>	20	6.2	55%

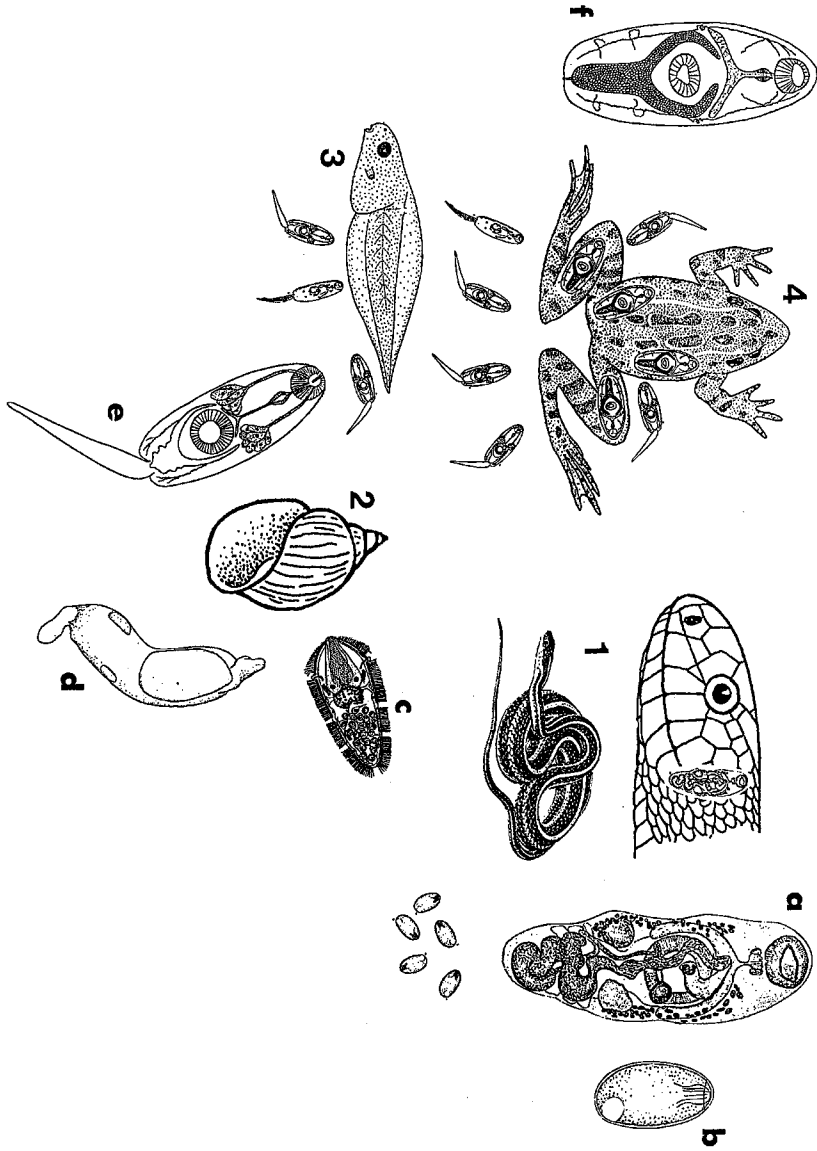


Plate 1. Life cycle of *Manodistomum syntomentera*. Adult worms (a) inhabit the roof of the mouth and esophagus of eastern garter snakes (1) (*Thamnophis sirtalis sirtalis*). Fully embryonated, operculated eggs (b) are shed in the feces of the snake and are presumably ingested by a snail first intermediate host (*Physa gyrina*) (2). The miracidium (c) hatches from the egg and develops into a sporocyst (d) within the snail. Free-swimming straight-tailed cercariae (e) emerge from the snail and penetrate into tadpoles (3) or adult frogs (4) to encyst as metacercariae (f) within the musculature or just under the skin.

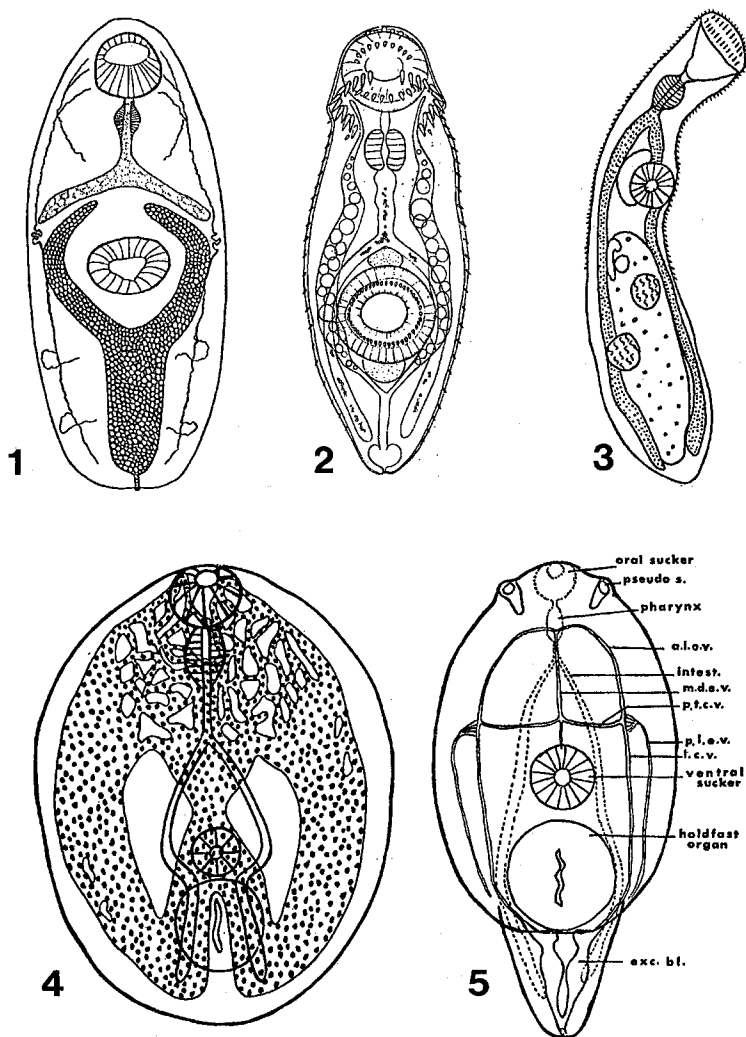


Plate 2. Metacercariae from frogs in the Mississippi River Valley Fig. 1. *Manodistomum* sp. Fig. 2. *Echinostome*. Fig. 3. *Heterophyid*. Fig. 4. *Diplostome*. Fig. 5. *Neascus*.