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Source: Journal of Wildlife Diseases, 24(4) : 647-649

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.4.647

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Journal of Wildlife Diseases, 24(4), 1988, pp. 647–649
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TRYPANOSOMES OF *BUFO AMERICANUS* FROM NORTHERN MICHIGAN

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ABSTRACT: Two hundred one American toads (*Bufo americanus*) from northern Michigan were examined for blood trypanosomes. Three species, *Trypanosoma bufophlebotomi*, *T. schmidti*-like sp. and *T. pseudopodia*, had prevalences of 27, 16 and 1%, respectively. Cross experimental inoculations showed that *T. bufophlebotomi* from toads is not the same as *T. ranarum* found in frogs of the family Ranidae of this region.

Key words: Michigan, American toad, blood parasites, *Trypanosoma bufophlebotomi*, *Trypanosoma schmidti*, *Trypanosoma pseudopodia*, *Bufo americanus*, field and experimental infections.

INTRODUCTION

Seven species of trypanosomes have been reported from the American toad (*Bufo americanus*) in the Great Lakes/St. Lawrence River region of North America. Fantham et al. (1942) described three new species of trypanosomes from Quebec, Canada. Another three species of trypanosomes were reported by Werner and Walewski (1976) and a seventh species was reported by Barta and Desser (1984).

The life cycles of these *Trypanosoma* spp. are unknown and the validity of some of them have been questioned. Of the three species described by Fantham et al. (1942), two (*Trypanosoma gaumontis* and *Trypanosoma lavalia*) were based on a single infection in a toad and the third species (*Trypanosoma montrealis*) was based on infections from two specimens of toads. None of these three species have been reported from toads since the original descriptions despite surveys in nearby areas (Woo, 1969a; Barta and Desser, 1984). Additionally, Barta and Desser (1984) concluded that Werner and Walewski (1976) had mistaken *Trypanosoma bufophlebotomi* from toads in Michigan's Upper Peninsula for *Trypanosoma ranarum*. *Trypanosoma bufophlebotomi* was described from *Bufo boreas halophilus* in northern California (Ayala, 1970), whereas *T. ranarum* is a common parasite of frogs of the families Ranidae and Hylidae in eastern North America (Woo and Bogart, 1984).

We report here additional collection records of trypanosomes from toads in Michigan's Upper Peninsula and experimental data to support the validity of *T. bufophlebotomi* from toads.

MATERIALS AND METHODS

Toads were collected by hand or dipnet from five general areas in Michigan's Upper Peninsula (Slapneck Creek, Alger County, 46°22′N, 86°57′W; Summit Lake, Baraga County, 46°43′N, 88°2′W; Lower Baraga Lake, Marquette County, 46°40′N, 88°2′W; Three Lakes, Marquette County, 46°35′N, 87°28′W; Driggs River, Schoolcraft County, 46°19′N, 86°7′W).

Blood (40 to 60 μl) was collected with a heparinized capillary tube after puncturing the facial vein at the angle of the toad jaw with a small gauge (22–26) needle. Trypanosomes were detected by four methods: wet mounts, thin smears, hematocrit tubes and smears of the hematocrit buffy layer (Woo, 1969b). Smears were fixed 1 to 5 min in methanol and stained with Giemsa stain.

Toads for experimental infections were collected at the Three Lakes site and maintained five to six per aquarium. Aquaria had several centimeters of wet sand and peat moss on the bottom. Toads were fed daily either mealworms, earthworms, fruit flies or crickets, depending on what was available. All toads were bled weekly and the blood was examined by the four methods listed above. Those toads which were negative by all methods for a 6-wk period were considered free of infection and used for experimental infections.

Due to the difficulty of obtaining typanosome-free frogs of the family Ranidae, laboratory-reared bullfrog tadpoles (*Rana catesbeiana*) were purchased (NASCO, 901 Janesville Ave., Fort Atkinson, Wisconsin 53538, USA) and

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used for experimental infections. *Rana catesbeiana* is a known host for *T. ranarum* in northern Michigan (Werner and Walewski, 1976) as well as other areas (Woo, 1969a). Tadpoles were maintained in groups of six in approximately 8 liters of lake water and fed a commercial tadpole food from NASCO (NASCO Frog Brittle). Blood was removed from the caudal vein. Individuals were bled weekly for 3 wk before inoculations to insure that they were not infected with trypanosomes (no infections were detected in over 40 tadpoles bled weekly for 8 wk using the above four methods).

For cross infections, *T. bufophlebotomi* was obtained from the toad (*Bufo americanus*) and *T. ranarum* from the green frog (*Rana clamitans*). Infected blood was removed by heparinized capillary tube from the facial vein or in some instances, animals were anesthetized in Tricaine Methanesulfonate (MS-222; Sigma Chemical Company, St. Louis, Missouri 63178, USA) and blood was removed from the heart with a heparinized syringe. The blood was mixed with a small amount of Amphibian Ringer's solution (Cable, 1977) and an approximate count of trypanosomes/ml was made by examining 5 μl under a coverslip. Using Ringer's solution as a diluent, between 500 and 2,000 trypanosomes in 0.1 cc were inoculated intraperitoneally into the experimental host. Control animals were inoculated with Ringer's solution only. Inoculations were repeated for 3 consecutive days.

Five test animals and a control comprised each experimental group. Cross infection experiments were accomplished by inoculating *T. bufophlebotomi* into bullfrog tadpoles and *T. ranarum* into toads. Simultaneously, positive toad blood was inoculated into uninfected toads and positive green frog blood was inoculated into bullfrog tadpoles to confirm the infectivity of blood forms of trypanosomes from one host to another. Experimental animals were bled weekly for 6 wk and blood was examined by the four methods listed above.

RESULTS

Field collections

Two hundred one toads were collected from 1982 to 1987. Fifty-four individuals were infected with *T. bufophlebotomi*, 33 with *T. schmidti*-like sp. and three with *T. pseudopodia*. Another seven toads were infected with a small trypanosome similar to *T. pipientis*. Probably this small form is a developmental stage of *T. schmidti* since four of the seven infected toads were found with the larger *T. schmidti* and the kinetoplast–nucleus relationships are similar between the two trypanomastigotes.

Experimental infections

Cross infection studies were performed in the summer of 1987. One individual died in each of the *T. bufophlebotomi*-inoculated toad and tadpole groups and they are not included in the results. The results showed that the two species were not cross-infective (none of five toads inoculated with *T. ranarum* became infected and none of four bullfrog tadpoles inoculated with *T. bufophlebotomi* became infected). Both trypanosome species were transmitted successfully between individuals of the same or similar host species as verified by stained smears (four of four toads inoculated with *T. bufophlebotomi* became infected and four of five bullfrog tadpoles inoculated with *T. ranarum* became infected). Trypanosomes were detected in the blood of infected animals between the second and fifth week and remained throughout the 6-wk period. The intensity of infection ranged from one to seven trypanosomes per μl of blood.

DISCUSSION

Our failure to find a previously described species, *T. pseudopodia* (Werner and Walewski, 1976) in toads from localities outside of the area where it was originally described, prompted a re-sampling of the original locality (Lower Baraga Lake). Again the parasite was found in two of eight toads. An additional infected specimen was found later at a site approximately 30 km south of the above original locality but on the same river drainage (Michigamme River, Michigan, USA). Since toads have not been sampled west of these localities, it is possible that the range of this trypanosome is more extensive.

The experimental infections indicated that *T. bufophlebotomi* found in toads of this region is not *T. ranarum* found in ranids. *Trypanosoma ranarum* has been reported as being pleomorphic; there are two trypomastigote forms, a slender and

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cornucopia form (Types I and II of Diamond, 1965). *Trypanosoma bufophlebotomi* shows a strong similarity to Type I but differs in two aspects: the body length is much shorter and the nucleus is located in the posterior one half of the organism in contrast to the anterior one third as seen in *T. ranarum*. *Trypanosoma bufophlebotomi* does not show the same similarity to the Type II form (see below). Diamond (1965) regarded the Type II as the most common trypomastigote form, Type I being rare in infections over 1 mo old. In our surveys, we have not observed a Type II form in over 200 toads examined, but we usually encounter the Type II form in ranids.

The trypanosome we have observed in toads (*T. bufophlebotomi*) is readily distinguished from the cornucopia (Type II form) of *T. ranarum*; the former is "C" shaped, non-costate and swims with a rapid corkscrew circular motion while the latter has a cornucopia shape, is highly costate and swims with a slow whole body undulating movement. It remains to be determined if *T. bufophlebotomi* of this region is actually the same parasite as *T. bufophlebotomi* described from toads in California.

Barta and Desser (1984) stated that their finding of *T. ranarum* in the wood frog (*Rana sylvatica*) was a new host record for this species. However, *T. ranarum* was reported earlier in *Rana sylvatica* by Werner and Walewski (1976). Barta and Desser (1984) also indicated that *T. ranarum* was originally described in European toads. However, *T. ranarum* was first described from the European common frog (*Rana esculenta*) (Lankester, 1871; Danilewsky, 1885), and to our knowledge this species has not been reported from the bufonids (toads) of Europe or elsewhere.

ACKNOWLEDGMENT

This study was supported by a Northern Michigan University Faculty Grant to the senior author.

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Received for publication 14 December 1987.

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