Widespread trypanosome infections in a population of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Virginia, USA

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ABSTRACT

Eastern hellbender salamanders (*Cryptobranchus alleganiensis alleganiensis*) are declining in North America, and because of this, the health status of individuals in several populations is closely monitored by researchers. During a health survey of hellbenders from a stream in Smyth County, VA, USA we examined giemsa-stained blood smears of 71 animals captured during 2011 for the presence of blood parasites. We discovered an unknown species of trypanosome that was apparently widespread within this population; 40 of the 71 individuals (56.3%) were infected. There are seven known trypanosome species of caudate amphibians; based on microscopic examination, the parasite we observed appeared most similar to *Trypanosoma cryptobranchi*, which was reported in this species only once before, 76 years ago, from a single animal apparently captured in Iowa. Given that some trypanosomes can adversely affect the health of their hosts, we recommend further monitoring be done in this and other hellbender populations to determine the geographic extent of the parasite and its effects on its increasingly rare host.

Key Words: Hellbenders, *Cryptobranchus alleganiensis*, trypanosome, *Trypanosoma cryptobranchi*

INTRODUCTION

In the eastern United States, populations of hellbenders (*Cryptobranchus alleganiensis*), a large aquatic salamander that lives in rocky streams, are declining (Wheeler et al. 2003; Foster et al. 2009; Burgmeier et al. 2011a). This is true for the eastern form (*C. a. alleganiensis*) which has a range encompassing much of the eastern United States and the Ozark hellbender (*C. a. bishopi*), which is restricted to a narrow range in the State of Missouri. In fact the Ozark hellbender was recently classified as an endangered species by the United States Fish and Wildlife Service (Federal Register 2011). Because of these population declines, numerous investigations have evaluated the health status of individuals from populations of both subspecies. These include examination of blood cell counts and morphology, hematology and serum chemistry, heavy metal loads, sperm quality, stress hormone levels, immunological responses, disease testing, and blood parasite prevalence (Jerrett and Mays 1973; Solis et al. 2007; Huang et al. 2010; Burgmeier et al. 2011b; Hopkins and DuRant 2011). With regard to blood parasites, in most cases few have been reported. One notable exception is a recent report of unidentified blood parasites from Ozark hellbenders (Huang et al. 2010), which appeared to be trypanosomes (J. Briggler, *pers. comm.*), although they were not examined in detail in that study. Recently, we examined blood films from a population of eastern hellbenders from Virginia, and discovered infections of trypanosomes; we describe these parasites in this paper?.

There are seven species of trypanosomes that have been reported in caudate amphibians (Woo and Bogart 1986). Only one has been reported in hellbenders; in a very

early account, blood from two eastern hellbenders was examined and one individual was infected with a trypanosome species (Roudabush and Coatney 1937). Roudabush and Coatney provided basic morphological measurements as well as line drawings and named the parasite *Trypanosoma cryptobranchi*. Later, Lehmann (1959) examined the same blood film and provided further details about the morphology of *T. cryptobranchi* and how it is distinguished from other trypanosomes in salamanders. A key characteristic is the appearance and position of the kinetoplast, which varies in shape and placement among species of salamanders, as it does across other vertebrate taxa (Clark and Wallace 1960). In *T. cryptobranchi* it is rod-shaped or elliptical, and located near the posterior end (15% of the body length from it). Another key characteristic is the centrally-located nucleus and the lack of endosome (Lehmann 1959). In the two other trypanosome species that have rod-shaped kinetoplasts (*T. granulosae* and *T. ambystomae*), the nucleus is not centrally-located in the body, and in *T. granulosae*, the endosome is consistently visible. Furthermore, Roudabush and Coatney (1937) stated that the kinetoplast in *T. cryptobranchi* is typically perpendicular to the long axis of the parasite body.

MATERIAL AND METHODS

Hellbenders were captured between June and August, 2011 from a stream in Smyth County, VA, USA as part of an ongoing effort to monitor the health of hellbenders in this location. The animals were all captured by hand (i.e. snorkeling) from under rocks and processed in the field, which included basic morphometrics, determining sex (based on the presence or absence of the swollen cloaca of males), implanting a pit tag for later

identification, as well as taking a blood sample. For this, blood was drawn from the caudal artery with a 26 gauge needle, and a drop was smeared on a clean microscope slide for blood parasite screening. Slides were air-dried and returned to the lab.

In the lab all slides were stained with a buffered wright-giemsa stain (Camco Quik Stain II) and examined with a light microscope, at 400X, by a single observer (AKD). For each slide, 50 random fields of view were examined for the presence of blood parasites. If no parasites were found, the animal was assumed to be uninfected.

RESULTS AND DISCUSSION

We examined blood films from a total of 71 hellbenders.. We were able to confirm the sex of63individuals; 31 were females and 32 were males. We noted trypanosome infections in 40 hellbenders (56.3%) overall. Seventeen females (54.8%) were infected while 19 males (59.4%) were infected with trypanosomes. At the magnification used (400X), the average number of trypanosome parasites observed in all infected animals was 2.0 (3.1 SD) in 50 fields of view (range = 1-14). Parasitemia level was not statistically different between males and females (two-sample t-test, df = 34, t = -1.56, p = 0.126).

Based on our observations and measurements, the parasite appeared most similar (with some exceptions, indicated below) to the description of *T. cryptobranchi* given by Roudabush and Coatney (1937). It was elongate, often with a corkscrew-like appearance, was bluish-purple under giemsa staining, had a rod-shaped, pink-staining kinetoplast located near the posterior end, and a centrally-located nucleus (Fig. 1A-E). In most cases

the long axis of the nucleus was oriented parallel to the longitudinal axis of the trypanosome body, whereas the kinetoplast was perpendicular to the long axis of the parasite body. The parasites measured an average of 40.7µm in length (range = 30.5-49.5, SD = 4.8), and 2.3µm in width (range = 1.8-2.9, SD = 0.3), based on measurements of 30 organisms. This is smaller than that reported by Roudabush and Coatney (1937), where the average length was 60.9µm (range = 46.8-77.4), and average width was 3.5µm (range = 1.8-5.9). Moreover, these authors reported a distinct broad undulating membrane and free flagellum (Fig. 1F), whereas we could not discern a flagellum on the parasites we observed, and a membrane was rarely seen (Fig. 1B). Also of note was the lack of variability in parasite appearance, either within individual blood films, or across all infected salamanders. Roudabush and Coatney (1937) indicated that at least three forms were present in the infected hellbender they examined; there were thin as well as stout specimens (Fig. 1F), whereas all forms in the current study were long and slender (Fig. 1A-E).

Given the precarious conservation status of eastern and Ozark hellbenders (Wheeler et al. 2003; Foster et al. 2009; Burgmeier et al. 2011a), any pathogen that could negatively affect their populations should be closely monitored. While there is no information on the pathogenicity of *T. cryptobranchi*, in other vertebrates some (but not all) trypanosome infections can negatively impact individual health (e.g., Muhammad et al. 2007). For example, an outbreak of *T. evansi* killed 51% of a population of horses in Brazil (Silva et al. 1995), *T. vivax* is known to cause anemia and weight loss in dairy cattle (Silva et al. 1999), and in humans *T. cruzi* is a principal cause of morbidity and mortality on the South American continent (e.g., Kayama and Takeda 2010), while *T.*

brucei is a debilitating infection in Africa (e.g., Smith et al. 1998). Furthermore, even if outward health effects are not apparent, subclinical infections could predispose individuals to increased mortality (Smith et al. 2008), reduced fecundity (Goossens et al. 1997) or reductions in immunocompetence (Rurangirwa et al. 1983). We also note that this represents the first reported case of trypanosome infections that we are aware of from the eastern part of the range of *C. a. alleganiensis*. While Roudabush and Coatney (1937) did not report where their infected hellbender was captured, they indicated it was caught locally and these authors were located in Ames, IA, which historically contained small populations of eastern hellbenders (Bishop 1943) and is the apparent western limit of the range of this species. The discovery of this parasite in the eastern portion of the hellbender range emphasizes that further monitoring must be done to determine if this pathogen is spreading among hellbenders and whether it adversely influences their health and survival.

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REFERENCES

Bishop SC (1943) Handbook of salamanders: the salamanders of the United States, of Canada, and of Lower California. Comstock Publishng Co., Ithaca, New York.

Burgmeier NG, Unger SD, Sutton TM, Williams RN (2011a) Population Status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. J Herpetol 45: 195-201.

Burgmeier NG, Unger SD, Meyer JL, Sutton TM, Williams RN (2011b) Health and habitat quality assessment for the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana, USA. J Wildl Dis 47: 836-848.

Clark TB, Wallace FG (1960) A comparative study of kinetoplast ultrastructure in the Trypanosomatidae. J Protozool 7: 115-124.

Federal Register (2011) Endangered and threatened wildlife and plants; endangered status for the Ozark hellbender salamander. Federal Communications Commission. 76: 61956-61978.

Foster RL, McMillan AM, Roblee KJ (2009) Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York state. J Herpetol 43: 579-588.

Goossens B, Osaer S, Kora S (1997) Long-term effects of an experimental infection with *Trypanosoma congolense* on reproductive performance of trypanotolerant Djallonke ewes and West African Dwarf does. Res Vet Sci 63: 169-173.

Hopkins WA, DuRant SE (2011) Innate immunity and stress physiology of eastern hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality. Gen Comp Endocrinol 174: 107-115.

Huang CC, Xu Y, Briggler JT, McKee M, Nam P, Huang YW (2010) Heavy metals, hematology, plasma chemistry, and parasites in adult hellbenders (*Cryptobranchus alleganiensis*). Environ Toxicol Chem 29: 1132-1137.

Jerrett DP, Mays CE (1973) Comparative hematology of the hellbender, *Cryptobranchus alleganiensis* in Missouri. Copeia 1973: 331-337.

Kayama H, Takeda K (2010) The innate immune response to *Trypanosoma cruzi* infection. Microbes Infect 12: 511-517.

Lehmann DL (1959) *Trypanosoma granulosae* n. sp. from the newt, *Taricha granulosa twittyi*. J Protozool 6: 167-169.

Muhammad G, Saqib M, Sajid MS, Naureen A (2007) *Trypanosoma evansi* infections in Himalayan black bears (*Selenarctos thibetanus*). J Zoo Wildl Med 38: 97-100.

Roudabush RL, Coatney GR (1937) On some blood protozoa of reptiles and amphibians. T Am Microsc Soc 56: 291-297.

Rurangirwa FR, Musoke AJ, Nantulya VM, Tabel H (1983) Immune depression in bovine trypanosomiasis: effects of acute and chronic *Trypanosoma congolense* and chronic *Trypanosoma vivax* infections on antibody response to *Brucella abortus* vaccine. Parasite Immunol 5: 267-276.

Silva R, Arosemena NAE, Herrera HM, Sahib CA, Ferreira MSJ (1995) Outbreak of trypanosomosis due to *Trypanosoma evansi* in horses of Pantanal Mato-grossense, Brazil. Vet Parasitol 60: 167-171.

Silva R, Ramirez L, Souza SS, Ortiz AG, Pereira SR, Davila AMR (1999) Hematology of natural bovine trypanosomosis in the Brazilian Pantanal and Bolivian wetlands. Vet Parasitol 85: 87-93.

Smith A, Clark P, Averis S, Lymbery AJ, Wayne AF, Morris KD, Thompson RCA (2008) Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia penicillata* (Marsupialia : Potoroidae). Parasitol 135: 1329-1335.

Smith DH, Pepin J, Stich AHR (1998) Human African trypanosomiasis: an emerging public health crisis. Brit Med Bull 54: 341-355.

Solis ME, Bandeff JM, Huang YW (2007) Hematology and serum chemistry of Ozark and eastern hellbenders (*Cryptobranchus alleganiensis*). Herpetologica 63: 285-292.

Wheeler BA, Prosen E, Mathis A, Wilkinson RF (2003) Population declines of a long-lived salamander: a 20+-year study of hellbenders, *Cryptobranchus alleganiensis*. Biol Cons 109: 151-156.

Woo PTK, Bogart JP (1986) Trypanosome infection in the salamanders (order: Caudata) from eastern North American with notes on the biology of *Trypanosoma ogawai* in *Ambystoma maculatum*. Can J Zool 64: 121-127.

FIGURES

1. Photomicrographs of trypanosomes (presumed to be *Trypanosoma cryptobranchi*) observed in eastern hellbenders from Virginia (A-E) as well as line drawings from the original description of *T. cryptobranchi* (F) from Roudabush and Coatney (1937). Small arrows point to the kinetoplast, which is rod-shaped or elliptical in *T. cryptobranchi.* Also note the centrally-located nucleus in all cases. The free flagellum was never seen, and an undulating membrane was rarely seen in the Virginia specimens (thick arrow in B).