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Loss of Trematode Parthenitae in *Planorbella trivolvis* (Mollusca: Gastropoda)

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ABSTRACT: Infection by trematode parthenitae (larval, asexual trematodes) has severe consequences for molluscan hosts, resulting in cessation of reproduction and early mortality. Here we present evidence that the freshwater snail *Planorbella trivolvis* can lose infections by trematode parthenitae. Of 8 *P. trivolvis* infected by reniferin parthenitae, 6 died within 2 wk, whereas the remaining 2 snails lost their infections within 82 days after initial examination. This phenomenon might suggest that molluscs can resist established trematode infections (i.e., "self-cure") or at least out-survive some trematode parthenitae.

Molluscs are frequently infected by trematode parthenitae (Dillon, 2000). The results of these infections include reproductive effects ranging from reduced reproduction (Etges and Gresso, 1965) to complete castration (Lafferty and Kuris, 2009), as well as natural history changes, which include gigantism (Mouritsen and Jensen, 1994) and behavioral modification (Lowenberger and Rau, 1994). Because trematode infections have such wide-reaching effects, the ability of molluscs to resist initial infection has been studied extensively (Vanderknaap and Loker, 1990; Horak and Kolarova, 2005; Mitta et al., 2012), but the loss of trematode parthenitae post-infection has been comparatively neglected (but see a review of elimination of *Schistosoma* spp. infections in Etges and Gresso 1965). Here we report on the loss of infection by plagiorchiid trematode parthenitae in 2 freshwater snails, *Planorbella trivolvis* (Say, 1817).

Forty-eight adult *P. trivolvis* were collected on 15 June 2012 from a wetland in Tampa, Florida (28°09′49″N, 82°18′42″W). After collection, snails were divided among 300 ml specimen cups containing artificial spring water (ASW; Cohen et al., 1980) into densities of 5 snails per cup and fed frozen spinach. Twenty-four hr later, snails were examined under a dissecting microscope for the presence or absence of free-swimming cercariae. Snails from cups containing cercariae were then individually observed 24 hr later for the shedding of cercariae. Cercariae from individual snails were preserved in 95% ethanol for sequencing (below). Infected snails were grouped by cercarial morphotype and maintained in the laboratory (23 C, 12:12 L:D) in 10 gallon aquaria containing ASW and fed frozen spinach ad libitum. *Planorbella trivolvis* does not venture above the water line (pers. obs.), so it is unlikely that uninfected snails could enter the aquaria of infected snails or vice versa.

Here 18S rDNA primers were used to amplify trematode DNA to (1) identify the cercariae shed from these snails and (2) assess whether the trematode infections were indeed completely lost by attempting to amplify any remaining trematode DNA in preserved snail tissue. DNA from approximately 100 armatae cercariae was extracted using an UltraClean® Fecal DNA kit (Mo-Bio Laboratories, Carlsbad, California). The primers BD1/4S and 3S/ITS2.2 from Wilson et al. (2005) were used to amplify the ITS-1 and ITS-2 regions. PCR was carried out in a total volume of 25 µl, which consisted of 1 µl of each primer (10 µM), 10 ng DNA, and 12.5 µl PCR Master Mix (Promega Corporation, Madison, Wisconsin). Reaction conditions were identical to Wilson et al. (2005). PCR product was viewed on an ethidium bromide-stained 1% agarose gel. DNA sequencing was conducted by Eurofins MWG Operon. Sequence results indicated 100% sequence similarity to both *Renifer aniarum* (Leidy, 1891) and *Lechriorchis tygarti* (Talbot, 1933). Because of the similarity to multiple species, cercariae are referred to as "armatae" hereafter. Both *R. aniarum* and *L. tygarti* belong to the subfamily Reniferinae in the family Plagiorchiidae (Byrd and Denton, 1938). Reniferin trematodes generally use similar host life cycles, utilizing freshwater snails as first intermediate hosts, tadpoles as second intermediate hosts (Talbot, 1933; Byrd, 1935), and snakes as definitive hosts (Talbot, 1934; Santoro et al., 2011). DNA was extracted from 95% ethanol-preserved snail tissue by first pulverizing tissue in liquid nitrogen, then using UltraClean® Fecal DNA kits (Mo-Bio Laboratories, Carlsbad, California). PCR was conducted using the 3S/ITS2.2 primer combination and the protocol described above.

Eight of the 48 snails screened shed armatae cercariae (16.7%), 4 shed furcocercous cercariae (8.3%), and 4 shed echinostomatid cercariae (8.3%) for a total prevalence of 33.3%. By 2 July 2012, although uninfected *P. trivolvis* housed in the laboratory exhibited good survivorship (exceeding 80%), the majority of infected snails were dead, and only 2 armatae-infected snails remained and both remaining snails shed cercariae. On 30 August 2012 these snails were examined under a dissecting scope together (i.e., not individually); the snails were not shedding cercariae. Furthermore, despite having laid no egg masses previously, one or both snails had laid 2 egg masses in their aquarium. Snails were not examined again until 4 September 2012, at which point one or both snails had laid 6 more egg masses, each of which contained more than 20 normally developing embryos, and again were not shedding cercariae. On 5 September 2012 the snails were dissected. These formerly trematode-infected snails had no visible evidence of trematode infection. Furthermore, PCR failed to amplify any trematode DNA, suggesting that the infections were completely eliminated.

We demonstrated that individuals of *P. trivolvis* naturally infected with plagiorchiid trematode parthenitae can lose the infection in the laboratory and resume reproduction within 82 days, whereas the remaining infected snails died during this time. Reports of clearing established trematode infections are uncommon and may not occur in all snail-trematode systems. Indeed, in well-studied systems such as echistomatid and *Fasciola* spp., loss of infection is unreported (Combes, 1982; Dillon, 2000), and this may suggest that loss of infections does not occur in these systems. *Helisoma anceps* was reported to eliminate infections by *Halipegus occidualis* (Goater et al., 1989), and among *Schistosoma*-infected snails, loss of trematode infections is considered rare, but does occur (Chu et al., 1966; Sturrock, 1967). However, increased mortality of *Schistosoma*-infected snails may obviate these effects (e.g., Barbosa et al., 1954; Dr. Fred Lewis, pers. comm.). This phenomenon of early mortality is substantiated by our own results, in which 75% of armatae-infected *P. trivolvis* died before any loss of trematodes could be observed. In the *P. trivolvis*–reniferin trematode system, infections can reduce laboratory survival despite uninfected snails having survivorship that exceeds 90% (Rohr et al., 2008).

If the loss of trematode infections in this and other snail-trematode systems is driven by host immunological responses, then elimination of trematode infections could have several ramifications in host-parasite dynamics. Immunologically based elimination might be a heritable trait, and snails might be capable of acquired resistance to trematode infections (e.g., Lie et al., 1983). Elimination of infection could, therefore, contribute to immunological memory and resistance against future infections. However, if snails are capable of eliminating trematode parthenitae only late in infection, at which point the majority of infected snails would have died, hosts might have little remaining reproductive value. Furthermore, it is possible that the loss of infection is not due to a directed immunological

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response from infected snails, but simply a result of parasite senescence. Few trematodes have been studied intensively enough to assess whether snails can eliminate patent infections; therefore, we encourage researchers to re-examine previously patent snails for lost infections.

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