Study Report

Does the novel parasite species that infects *Helisoma* snails cause swimmer's itch?

Study funded by the Higgins Lake Swimmer's Itch Organization (HLSIO)

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Introduction

In 2021, the HLSIO funded our research proposal to study whether a novel schistosome parasite is a major or minor cause of swimmer's itch. The novel schistosome was recently discovered in *Helisoma* snails and found to use the Canada goose as the waterfowl host. While it had been recently shown that the parasite could cause swimmer's itch (McPhail et al. 2021) the study was very small (1 out of 4 people developed a single papule).

Here, we report the results of a much larger and more comprehensive study that includes a side-by-side comparison of the known cause of swimmer's itch on Higgins, Crystal, Glen, Lime, Douglas, and Black lakes (among others): *Trichobilharzia stagnicolae*, which uses *Stagnicola* snails as hosts and the common merganser as the waterfowl host.

The results show that the novel schistosome from *Helisoma* snails (hereafter named HAS, for *Helisoma* avian schistosome) is much less effective than *T. stagnicolae* at penetrating human skin and resulting in papules – at least 40X less effective. We have been writing this up as a scientific paper to be submitted for publication, and the next two sections (Materials and Methods, Results) are from that developing manuscript and contain many details. You can skip these two sections and read the summary points if desired.

Methods and materials

Snail collection, isolation, and housing. Infected *S. emarginata* and *P. trivolvis* snails were collected by wading or snorkeling in June and July 2021 in Black Lake, Larks Lake, and Walloon Lake, MI, and North and South Twin Lakes, WI. Snails were housed in complete darkness overnight in buckets with fresh lake water, and in the morning, snails were individually isolated in well water and exposed to light for at least 1 hour to stimulate the emergence of cercariae (Blankespoor and Reimink 1998). Cercariae visually identified as avian schistosomes were preserved in DNA-grade ethanol and the host snails were transferred to aquaria for long-term maintenance. Snails were housed in 20L plastic bins filled with artificial pond water. Undergravel filters were covered by a 1.5 cm layer of gravel followed by 3 cm layer of sand from collection sites. Snails were fed lettuce *ad libitum* by embedding the stalk in the sand, and detritus was siphoned from the bottom biweekly.

DNA extraction and sequencing. We extracted DNA from the preserved cercariae (Qiagen DNEasy blood/tissue kit) and used published primers to amplify regions of the 18S rRNA by PCR (primers

18JVSQF, 18SJVSQR), 28S rRNA (28SJVSQF, 28SJVSQR, C1, D2), and COI genes (HCO,LCO). PCR products were purified with Exo-SAP-IT and submitted for Sanger sequencing.

Volunteer recruitment. Student and faculty volunteers at Calvin University were recruited for the study via an announcement on email listservs. Interested candidates were asked to fill out a 10-item questionnaire requesting age, gender, health and allergy information, prior swimmer's itch exposure, and inland lake swimming history. Candidates were excluded from the study if they had a health or allergy condition that might increase their risk of a severe reaction. Study participants received a $50 stipend at their follow-up appointments.

Exposure procedures. Aquaria of infected snails were covered overnight to prevent premature light exposure. In the morning, snails were isolated in untreated 6-well tissue culture plates and exposed to light for 1 hour. Emergent cercariae were then used in human exposures at 1-6 (mean 2.9 ± 1.5) hours after snails were first exposed to light. Preliminary observations of the HAS parasite indicated that they are especially prone to adhere to surfaces with their oral and ventral suckers and were more likely to be stuck to Pasteur pipettes than *T. stagnicolae*. In order to reduce the rate at which both species stuck to pipettes, we used glass pipettes siliconized with Sigmacote®. In addition, using a dissecting microscope we deposited small numbers of cercariae onto a watch glass, so we could accurately determine the number of cercariae drawn into the pipette before transferring it onto participant skin.

Study participants were asked to sit between two microscopes with their forearms supine on the table in front of them. We used siliconized glass pipettes to transfer 5 droplets of water containing cercariae onto each arm. Droplets containing *T. stagnicolae* were placed on the left arm and HAS on the right. After each droplet transfer, the pipette was checked under the microscope to ensure the cercariae had not stuck inside the pipette. In the first four exposures, every HAS droplet contained one cercaria each. In all remaining exposures, to increase the number of opportunities for HAS cercariae to penetrate, each droplet contained 2-3 HAS cercariae. Droplets containing *T. stagnicolae* always contained a single cercaria each. One exposure had only 4 droplets of HAS cercariae because of insufficient cercariae. Study participants remained seated for 45-60 minutes to allow droplets to evaporate completely.

Study participants returned for follow-up observation 24-72 hours later. The number of papules on each arm were recorded, and non-identifying photos were taken (e.g., Figure 1). All participants were asked if they experienced any itching, when they first noticed papules appearing, whether papules had increased or decreased in size over time, and if they applied any anti-itch creams. To determine if prior exposure might increase the number of responses to HAS cercariae, participants were also asked if they were interested in a second exposure. Eight participants agreed to be exposed a second time 11-20 days after their first exposure and returned for follow-up observation. Calipers were used to measure papule diameters for 10 participants. A Wilcoxon signed-rank test was used to determine whether the two species differed in the number of papules produced.

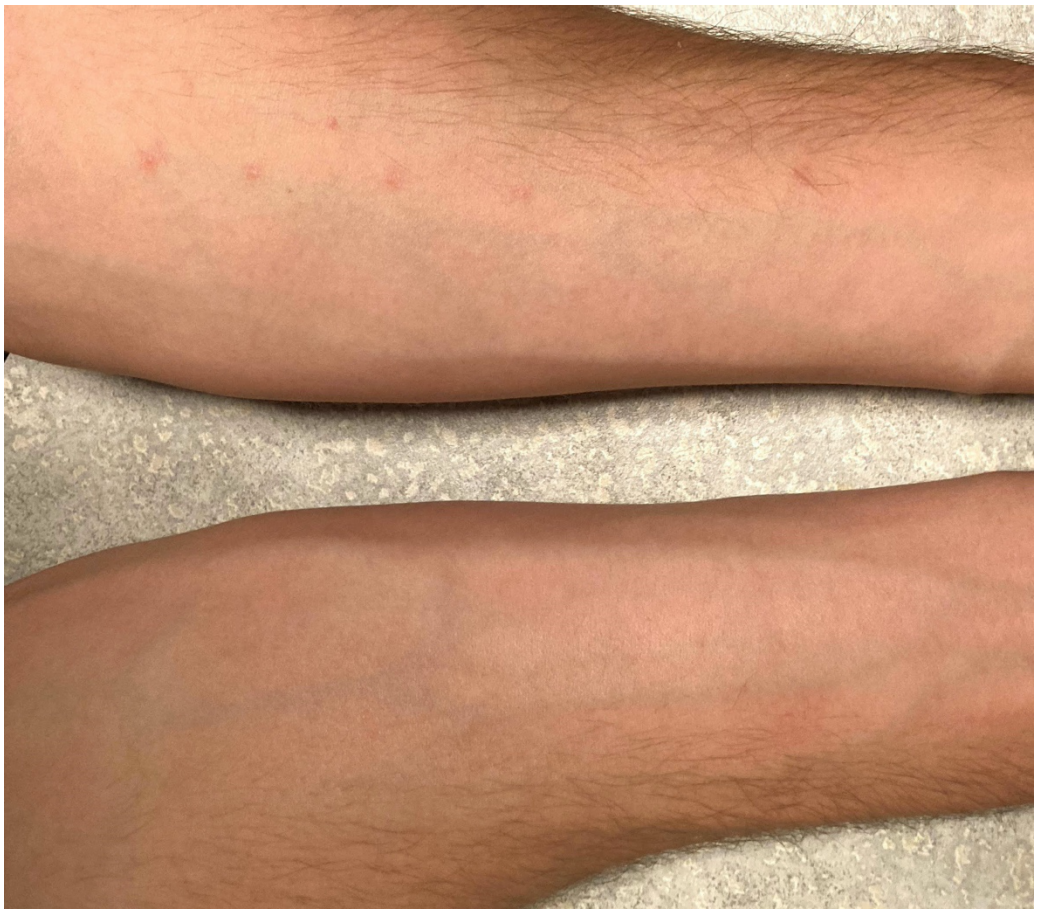

Figure 1. Arms of volunteer at post-exposure follow-up visit. Left arm (top), which was exposed to 5 *T. stagnicolae* cercariae, has 4 visible papules. Right arm (bottom), exposed to at least 10 HAS cercariae, did not develop any papules.

Results

Striking differences were observed between the two species. In the first exposure, 16 of 24 study participants showed reaction to *T. stagnicolae,* developing 1-5 papules after their first exposure (median 2.0 ± 1.5 interquartile range). Only one individual had a single HAS papule, along with 2 *T. stagnicolae* papules. Eight individuals who had developed papules to *T. stagnicolae* in the first exposure volunteered for the second exposure. Of these, 7 developed 1-4 papules to *T. stagnicolae* (median 1.0 ± 1.5 interquartile range) in the second exposure. Only one second exposure volunteer developed a HAS papule, along with 2 *T. stagnicolae* papules.

Each cercaria pipetted onto an arm can be viewed as an opportunity for penetration and papule development, and analyzing the data this way shows conspicuous contrast. In total, *T. stagnicolae* produced a significantly higher number of papules (49 of 160, 30.6%) than HAS cercaraiae (2 of 303, 0.7%; p=0.000000217, Wilcoxon signed-rank test). In other words, the *T. stagnicolae* cercariae were 43X more likely to penetrate and result in a papule than the HAS cercariae (Figure 2). Papule size ranged from 1.2 to 12.0 mm for *T. stagnicolae* (n=49, mean 3.61 ± 2.77 mm) and the two HAS papules were 1.4 and 8.2 mm.

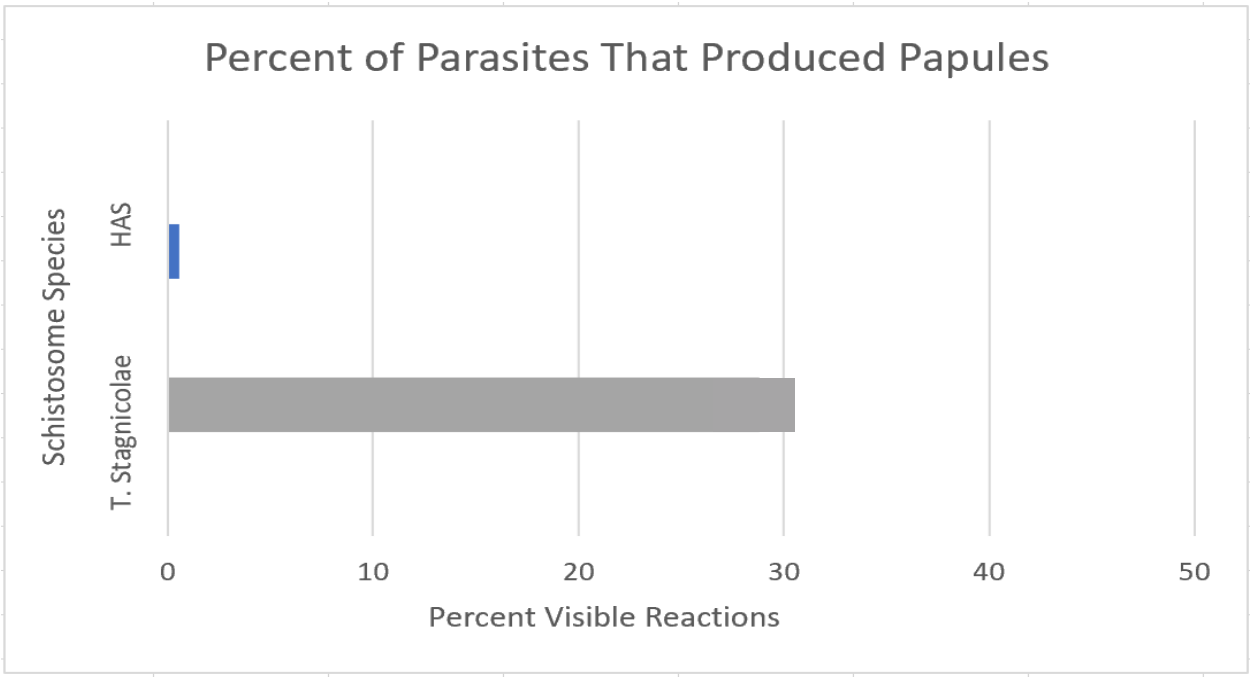

Figure 2. Percentage of schistosome parasite cercariae that induced papules in 32 exposures of human volunteers. A species known to cause swimmer's itch papules, *T. stagnicolae,* did so for 49 out of 160 cercariae (30.6%). The lesser known species, HAS, did so for 2 out of 303 cercariae (0.7%).

Most individuals who developed papules reported some itching, usually within the first few hours after exposure, before the appearance of papules, and ending at 6-24 hour after exposure. Of the 16 individuals developing *T. stagnicolae* papules in the first exposure, 14 reported itching, all on the *T. stagnicolae* exposed arm only. Two of the 8 individuals who did not develop papules reported brief mild itching. The individual who developed papules from both species reported itching on both arms. Of those individuals who underwent a second exposure, 5 of 7 who developed *T. stagnicolae* papules reported itching on the *T. stagnicolae* exposed arm only. The individual who developed papules from both species in the second exposure reported itching for the arm exposed to *T. stagnicolae* but not the arm exposed to HAS.

Summary points:

Main result: Our results show that HAS cercariae are far less likely to produce papules than *T. stagnicolae* (0.7% vs. 30.6%, a difference factor of 43X). Only 2 people got one papule each from HAS cercariae, whereas 23 out of 32 people got 1-5 papules from *T. stagnicolae*. These contrasting results are due to a very low rate of human skin penetration for HAS cercariae.

This is a robust study: 32 total volunteer exposures, with totals of 160 *T. stagnicolae* cercariae and 303 HAS cercariae used.

8 of the exposures were volunteers who were exposed twice. Results were very similar for this group in the second exposure, indicating that re-exposure to HAS cercariae was not critical for papule formation.

While the difference between the species could be in part an artifact of the exposure method and it is impossible to exactly mimic 'natural' contact between the parasites and a swimmer, we don't think this is likely the source of the difference. Such a large difference is likely to exist in 'natural' conditions too. In fact, it is possible that the difference could be even greater with natural contact (i.e., *T. stagnicolae* penetration success might be higher under natural conditions while HAS penetration success remains low; see also the additional investigations below).

This is the first study we are aware of to show a strong difference in the penetration behavior of avian schistosome cercariae from different species. That will be one of the messages of the published paper: **not all avian schistosome species are the same in their propensity to cause swimmer's itch!** Nor should we expect all species to be the same, since they each infect different waterfowl hosts and the adaptations they have for those hosts may or may not make them likely to penetrate human skin.

Additional questions and tests:

Ongoing 'exposure': the infected *Helisoma* snails from this summer have been housed in the DeJong lab with some still alive in November! Randy has been making a weekly practice during that time of dipping his full forearm into this tank, and never gets a papule! If he did this in a tank of infected *Stagnicola* he would get papules every time.

Is the HAS cercariae 'weaker' and therefore less able to penetrate human skin? HAS cercariae are smaller, and in tests of longevity did run out of energy and become immobile more quickly than *T. stagnicolae* cercariae.

Is the HAS cercariae just not 'as interested' in penetrating human skin? We also did some preliminary studies in the lab that showed that HAS cercariae did not react nearly as strongly to linolenic acid, a fatty acid from human skin previously identified as a strong stimulant for cercariae penetration. In contrast, *T. stagnicolae* cercariae were very stimulated to penetrate by linolenic acid as predicted.

Perhaps the HAS cercariae does not even penetrate goose skin, but enters the Canada goose another way? We obtained a Canada goose from a hunter and hypothesized that the HAS cercariae might enter through the esophagus when the goose drinks or feeds. We dissected the esophagus and placed cercariae of the two species in the interior, which is much softer tissue than human skin. It is very difficult to see the cercariae on the tissue, so our numbers are low, but a high percentage of the HAS cercariae penetrated (13 of 16) and none of the *T. stagnicolae* (0 of 13)! We will have to see whether we have the opportunity to do this again, as it depends on getting another Canada goose and the snails continuing to survive in the lab.

Conclusion

The novel schistosome from *Helisoma* (HAS) appears to be much less capable of causing swimmer's itch than *T. stagnicolae.* The substantial difference in penetration and induction of papules is probably a contributing reason that HAS went such a long time without being discovered, despite the wide range and commonness of both the snail (*Helisoma*) and waterfowl (Canada goose) hosts. It is possible that the HAS species may play a minor role to swimmer's itch in some lakes where the correct snails and Canada goose are found, but it is very unlikely to be the majority cause of severe swimmer's itch problems. For lakes that have both species present, the findings reported above suggest that trap and relocation of common merganser broods would have a strong impact on swimmer's itch incidence and severity.

Regulatory approvals: The protocols for recruiting and exposing human volunteers were reviewed and fully approved by the Institutional Review Board (IRB) of Calvin University. The use of hunted Canada goose was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Calvin University.

References

Blankespoor, H. D., and R. L. Reimink. 1998. "An Apparatus for Individually Isolating Large Numbers of Snails." *The Journal of Parasitology* 84 (1): 165–67. https://doi.org/10.2307/3284550.

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