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TREATMENT AND PROPHYLAXIS - SHORT COMMUNICATION

Evaluation of targeted copper sulfate ($CuSO_4$) application for controlling swimmer's itch at a freshwater recreation site in Michigan

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Abstract

Swimmer's itch has historically been controlled by applying copper sulfate ($CuSO_4$) to lakes as a way to eliminate snails that serve as the intermediate hosts for swimmer's itch-causing parasites. $CuSO_4$ is still sometimes applied specifically to areas of lakes where swimmer's itch severity is high. It is unclear whether targeted application of chemical molluscicides like $CuSO_4$ is effective for controlling swimmer's itch. Previous research has found that the larval stage of the parasites responsible for swimmer's itch are released from infected snails and are concentrated by onshore and alongshore winds, and thus, may not be affected by such focused applications. In this study, we evaluated the impact of targeted $CuSO_4$ application to a specific recreational swimming area in a lake in Michigan. We measured the effect on snail populations, as well as on the presence/abundance of swimmer's itch-causing parasites using qPCR. Ultimately, while $CuSO_4$ was confirmed to significantly reduce populations of snails within the treatment area, it was found to have no significant impact on swimmer's itch-causing parasites in the water, likely due to the free-swimming larval stages (cercariae) moving into the treatment area from surrounding regions.

Keywords Swimmer's itch · Digenetic trematode · Schistosome · qPCR · Water · Copper sulfate · Chemical treatment

Introduction

Swimmer's itch, or cercarial dermatitis, is a skin irritation caused by larval avian trematodes of the family Schistosomatidae. These avian schistosomes use birds or mammals as their definitive hosts (Cort 1928). Most worms reside in veins surrounding the large intestine of their definitive host where the female worm produces eggs that exit via feces. A notable exception to this lifecycle is *Trichobilharzia regenti,* which resides in the nasal mucosa of anatid birds and sheds eggs via the nose when the bird host eats or drinks (Rudolfová et al. 2002; Marszewska et al. 2018; Horák et al. 1999). In an aqueous environment, the fully embryonated eggs hatch into free-swimming miracidia. Intermediate snail hosts, often from the genera *Stagnicola*, *Physa*, or *Gyralus*, become infected when miracidia penetrate soft body parts and develop into sporocysts within the hepatopancreas. Second-generation daughter sporocysts produce free-swimming, fork-tailed, ocellate cercariae asexually. Cercariae are shed from the snail each morning and migrate to the surface of the water in search of a suitable bird or mammal to complete their lifecycle (Rudko et al. 2018). A single infected snail can produce several thousand cercariae per day (Soldánová et al. 2016). These free-living avian schistosome cercariae can mistake other endothermic organisms, notably humans, as their target host. Upon penetration of the human epidermis, they are quickly destroyed by the immune system, leaving raised, itchy papules, which can last for weeks. Individuals who have experienced swimmer's itch multiple times can develop a hypersensitivity to cercariae (Olivier 1949). The condition is a nuisance to humans but rarely creates long-term health concerns.

Swimmer's itch has a storied history in Michigan. William W. Cort discovered the life cycle of these parasites at Douglas Lake (Cheboygan Co., MI) in 1928, prompting extensive research in the following decades. Once the lifecycle became

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better understood, different methods for controlling swimmer's itch began to emerge. One of the most popular was the treatment of swim areas/lakes with copper sulfate ($CuSO_4$), using it as a molluscicide targeting the snail intermediate hosts.

Initially, copper sulfate was used to control algae on recreational lakes beginning in the early 1900s (Hanson and Heinz 1984). It was used as a molluscicide in Michigan beginning in 1939 when the state began allowing $CuSO_4$ application on lakes with a documented problem of swimmer's itch (Blankespoor and Reimink 1991). More recently in the USA, molluscicidal $CuSO_4$ application has been used to control *Planorbella trivolvis*, the intermediate host of *Bolbophorus* sp., a trematode that causes increased mortality in farmed channel catfish (Wise et al. 2006). Additionally, $CuSO_4$ is also effective at killing free-swimming cercariae. Studies conducted on Echinostome cercariae found that concentrations as low as 0.01% killed 100% of cercariae within 2 h (Reddy et al. 2004). This research suggests copper sulfate should be an effective chemical for controlling swimmer's itch. Chemical controls on large recreational lakes may be ineffective, however, due to the relatively short toxicity period of Cu^+ ions before precipitation as $CuCO_3$ (< 24 h) and the planktonic nature of cercariae allowing wind and water currents to move and concentrate cercariae.

Using qPCR cercariometry, Rudko et al. (2018) demonstrated cercariae are concentrated towards shoreline areas during periods of along or onshore winds. qPCR cercariometry is a method of measuring planktonic cercariae in the water and is superior to traditional methods of swimmer's itch assessment which require the collection and shedding of thousands of snails. qPCR technology quickly measures the number of cercariae in a water sample and provides a direct assessment of schistosome cercariae abundance in the water, which can be a reflection of swimmer's itch risk for potential bathers. Using these new technologies, we tested the effectiveness of $CuSO_4$ at reducing planktonic cercariae in recreational water in a controlled and targeted application context to determine whether focal application of $CuSO_4$ actually reduces schistosome cercariae abundance at a swim site.

Our study site on South Lake Leelanau (Leelanau County, MI; Fig. 1) has a long, unbroken history of $CuSO_4$ application. Local riparians have been applying $CuSO_4$ annually for over 40 years, with only anecdotal evidence driving the justification for application. This location has a consistent history of swimmer's itch occurrence and Rudko et al. (2018) found hatch-year mallards (*Anas platyrhynchos*) infected with *Dendritobilharzia sp.* on South Lake Leelanau as well as common mergansers (*Mergus merganser*) infected with *Trichobilharzia stagnicolae* on North Lake Leelanau. *Stagnicola emarginata* and *Physa sp.* snails, both known hosts for avian schistosomes, inhabit the study site. Thus, all of the biological elements are present for swimmer's itch to be a persistent problem at this swim site.

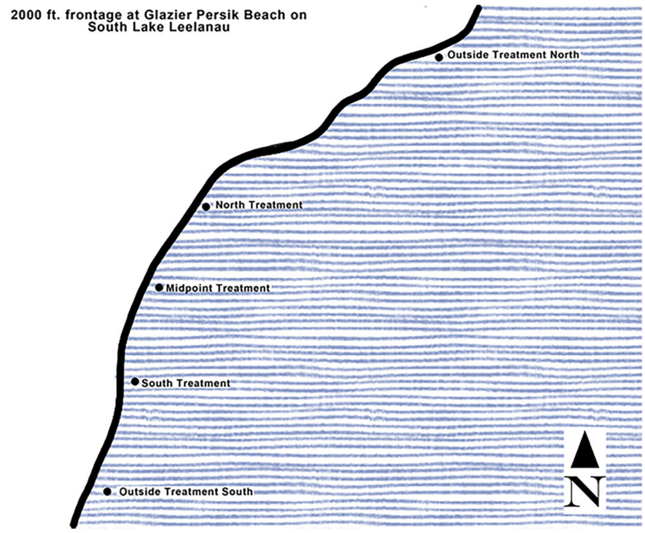

Fig. 1 Water sampling sites. The three red sites were treated with CuSO4, while the two yellow sites were control sites. Water samples were collected at each of the five sites pre- and post-treatment

Here we use qPCR cercariometry to quantify the number of cercaria in the water column before and after application of copper sulfate in an area with a well-documented swimmer's itch problem in order to ascertain if copper sulfate application is effective at controlling swimmers itch for recreators. Our data reinforce the effectiveness of $CuSO_4$ as a molluscicide but also show it does not significantly reduce the risk of contracting swimmer's itch as measured by estimating the number of cercariae in the water using qPCR cercariometry.

Materials and methods

Study site

South Lake Leelanau (SLL) is a mesotrophic lake with a surface area of 5932 acres and a mean depth of 7.2 m. A submerged riverbed connects it to North Lake Leelanau, which measures 2914.3 surface acres with a mean depth of 12.2 m. The combined shoreline of the lakes is about 66.3 km (Breck 2004). Our study area was in the Glazier-Persik community on the southwest shore of SLL. This lakefront runs approximately 625 m and is densely populated with residences used as year-round homes, summer homes and rental properties.

Three water collection sites were selected within the copper sulfate treatment area, one in the middle and one near each end. Two control sites were selected outside the treatment area, one 600 m to the north and one 300 m to the south, both far enough removed so no residual copper could impact the snails or cercariae (Fig. 1). Current flow and prevailing winds consistently move the planktonic community in the direction from south to north at this location.

Research was conducted in late June and July to assure water temperatures and photoperiods were conducive to cercariae shedding and to provide enough pre- and post-sampling dates for collecting statistically significant data (Table 1).

Treatment

A total of 90.13 kg of fine granular copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was applied evenly by a line of volunteers wading across the treatment area. Copper sulfate was applied by hand using small handheld scoops on the evening of June 25, 2017, under clear and calm conditions. Conditions remained calm and stable throughout the night and into the next day, well past the stage when the toxic copper had all precipitated as non-toxic $CuCO_3$.The area measured approximately 625 m long × 32 m wide with an average depth of 0.9 m. The total water volume within the treatment area was estimated at 18,200 m^3. The average copper concentration was 51.11 ppm, which is well above the recommended concentration of 20 ppm. Water alkalinity plays a role in how quickly copper precipitates as $CuCO_3$, but based on the alkalinity of most lakes in Michigan, lethal concentrations of the copper ion are expected to last for less than 24 h (Michigan Department of Environmental Quality 2014; O'Neal and Soulliere 2006).

Snail collections

Four days prior to the 25 June copper sulfate application, snail densities were determined for all snail species. Using snorkel equipment and wet suits, two researchers randomly tossed weighted 0.5 m^2 vegetation sampling hoops (Forestry Suppliers #78503) and recorded the number of snails of each species in each hoop. Each field biologist threw the hoop 200 times, which resulted in a total sample area of 200m^2. A zigzag pattern through the length of the treatment area assured all water depths were sampled. Only mature snails were counted. The identical procedure was repeated 10 days after copper sulfate treatment. Additionally, snail densities were assessed 50 m on either end of the application area to serve as a control, with a total of 50 m^2 sampled in each control zone.

Water collection

A standardized water collection and processing protocol was developed and used throughout. Fifty (50) 1 L water samples were drawn from an approximately 100 m^2 area for each collection. Water was passed through a 20 um mesh plankton net and the filtrate concentrated to approximately 25 ml. Ninety-five percent ethanol was added to bring the total volume to 50 ml. Samples were suction filtered through a 0.4 um filter (Pall, FMFNL 1050) and the filter cut in half. One-half filter was preserved in 95% ethanol and frozen at − 80 °C for future reference. The other half was used to extract DNA using the Biomeme DNA extraction kit (Biomeme M1 Sample Prep).

Pre-treatment water samples were collected at the three experimental and two control locations daily from June 19–23. The first week post-treatment, water samples were taken two different days (June 28 and 29) and then once a week for the next 4 weeks.

qPCR cercariometry

qPCR cercariometry was carried out according to Rudko et al. (2018) and Jothikumar et al. (2015). Briefly, 5 uL DNA extracted from filtered water samples was added to 15 uL of qPCR master mix containing 1X PrimeTime Gene Expression Master Mix (IDT), and 200 nm each of JVSF and JVSR primers, and probe, JVSP (Jothikumar et al. (2015). Thermocycling was performed using the ABI 7500 qPCR machine (Applied Biosystems), using a standard, 40 cycle, two-step reaction, which consisted of a 30 s hold at 95° followed by a 30 s denaturation cycle at 95° and a 60° annealing cycle.

Statistics

A two-way ANOVA was used to compare pre- and post-treatment cercariae abundance. A chi-square test was used to compare number of snails found on each side of the treatment area.

Table 1 Number of cercariae per 25 L sample as estimated by qPCR, both pre- and post-treatment

Site Location	19 June	20 June	21 June	22 June	23 June	$CuSO_4$	28 June	29 June	7 July	13 July	17 July	27 July
Outside N	0	0	305.16	0	0.69		0	0.92	0	N/A	0	4.31
Outside S	0	0	N/A	0	0		0	0	100.51	18.68	5.62	1.12
Inside A	0	0	88.64	0.74	26.90		26.33	0.51	0.85	5.22	12.17	0
Inside B	3.15	0	26.76	4.52	0		2.65	16.51	21.97	59.67	18.16	3.53
Inside C	0	0	4.71	4.47	0		2.36	0	96.35	30.76	1.20	1.59

Table 2 Total snail numbers and average snail densities (snails/m^2) found in entire treatment area pre- and post-treatment

Trial	*Stagnicola*	*Physella*	*Campeloma*	*Helisoma*	*Pleurocera*	*Martonia*
Pre-$CuSO_4$	14 (0.07)	122 (0.61)	89 (0.445)	95 (0.475)	150 (0.75)	55 (0.275)
Post-$CuSO_4$	1 (0.005)	14 (0.07)	4 (0.02)	22 (0.11)	307 (1.535)	10 (0.05)
% Change	− 92.86%	− 88.52%	− 95.51%	− 76.84%	104.67%	− 81.82%

Results/discussion

Copper sulfate applied at a concentration of 51.11 ppm in a single dose was effective at decreasing snail densities inside the treatment area (Table 2). Six species of snails were found in the study area prior to copper sulfate treatment. Each was identified by gross morphology. Snails from the genera *Stagnicola*, *Physella*, *Campeloma*, *Helisoma*, *Pleurocera*, and *Marstonia* were found. Counts of all species except for *Pleurocera sp.* decreased significantly after treatment ($p < 0.0001$, $df = 5$). Divers did not sample deeper than 1.5 m, yet it was noted that the deep side diver found significantly more snails than diver in the shallow area ($\chi^2 = 66.01$, $p < 0.00001$). The two snails that act as hosts for avian schistosomes, *Stagnicola sp.*, and *Physa sp.*, decreased by 93% and 89%, respectively (Table 2). As snail densities were counted post-treatment, all living *Stagnicola sp.* and *Physa sp.* were collected and brought back to the laboratory. A total of 23 *Physa sp.* and 1 *Stagnicola sp.* were collected, isolated, and shed for cercariae identification using the methods of Blankespoor et al. (1985). None showed patent infections.

Copper sulfate treatment was not effective at significantly reducing the number of cercariae in the surface waters within the treatment area (Table 1, Fig. 2, Fig. 3). On June 23 (pre-treatment), only the north inside treatment location had detectable numbers of cercariae in the water (26.9 cercariae/25 L). On the next sampling date, June 28, there was no change in cercariae number, we detected 26.3 cercariae/25 L, and at the midpoint inside and south inside sampling points, we detected 2 cercariae/25 L. After July 29, cercariae numbers continued to increase for all sites before beginning to decrease towards the end of the sampling period. (Fig. 3). This cyclic pattern of cercariae is consistent with previous findings both from water sample analysis and shedding snails (Fig. 3).

Several papers have demonstrated that cercariae are susceptible to killing by $CuSO_4$ (Reddy et al. 2004; Soucek and Noblet 1998). Our data suggest that cercariae released from snails outside the treatment area drifted into the treatment area for weeks after all copper had precipitated. Snails surrounding the treatment area were presumably not killed by copper sulfate due to concentrations below lethal levels (20 ppm). If these infected snails are shedding cercariae, these parasitic larvae could be moved to shallow waters by currents and surface winds. This corroborates the results of the generalized linear model presented in Rudko et al. (2018), which suggested that cercariae concentrations are

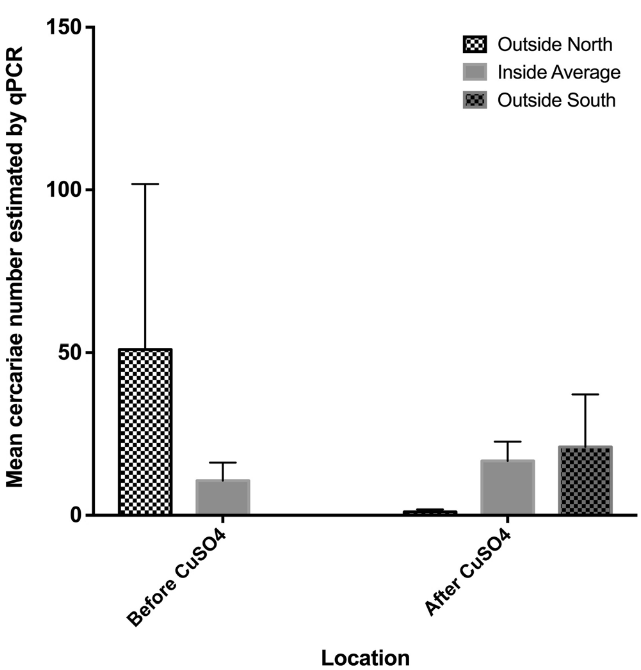

Fig. 2 Mean number of cercariae found in waters samples inside and outside of the treatment area both before and after treatment as found by qPCR

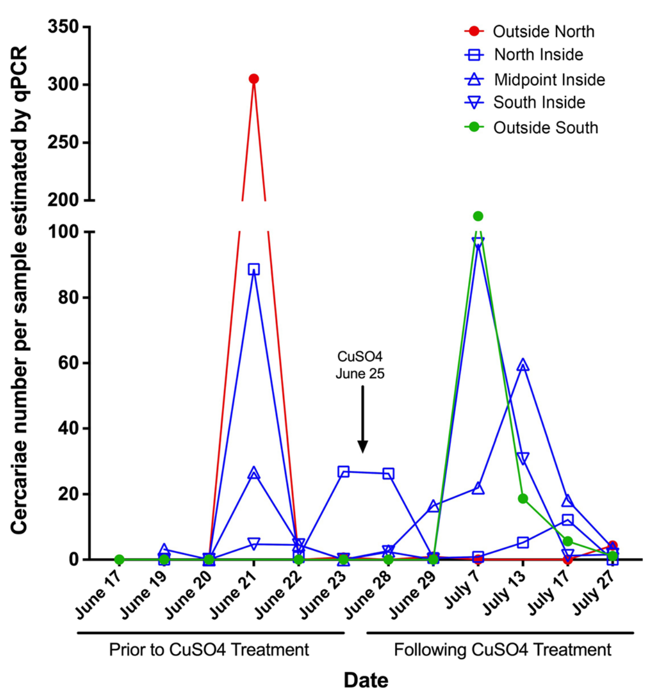

Fig. 3 Number of cercariae per 25 L sample as estimated by qPCR, both pre- and post-treatment. CuSO4 treatment occurred on June 25

influenced by surface winds. A few snails that survived copper sulfate treatment were collected and none harbored patent infections. This also supports the idea that cercariae measured in water samples originated from outside the treatment area.

Conclusion

This study suggests that $CuSO_{40}$ applied in local areas of a larger water body, while effective at reducing local snail populations and possibly the existing cercariae population, are, ultimately, ineffective at controlling the risk of swimmer's itch beyond the day of application. Planktonic schistosome cercariae move with the bulk flow of water and enter the treated area shortly after lethal concentrations are dissipated. Additionally, our study demonstrates the importance of utilizing qPCR cercariometry of ambient water to assessment swimmer's itch risk, since an absence of snails does not necessarily equate to the absence of cercariae present in the water.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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