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Research Article

Liquid Iodine and Liquid Chlorine as Novel Cost Effective Methods of Water Purification Through the Elimination of *Turbatrix Aceti* Nematodes

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Abstract

The overall purpose of this research study was to identify a cost effective method of preventing water borne roundworm infections. Such infections are prevalent in humid areas without access to proper water filtration systems. Varying concentrations of liquid iodine and liquid chlorine were mixed with apple cider vinegar and diluted with water to simulate the conditions of unfiltered water containing microbes that *Turbatrix aceti* would feed on. This was then used to create solution agents that were then tested on groups of 10 *Turbatrix aceti*. Eventually it was determined that solutions composed of .0667% liquid iodine and .2% liquid chlorine would be capable of eliminating 100% of the Turbatrix aceti trial groups. Additionally, solutions containing .0333% liquid iodine and .10% liquid chlorine were developed to eliminate half of each group. These four solutions were tested ten times each, which resulted in the 100% death solutions eliminating all roundworms. The intended fifty percent death groups or for iodine and chlorine eliminated 55% and 54% of the roundworms on average. The overall p-value was found to be 3.96 * 10-20 which was far below the alpha value of .05, demonstrating significance. Following a Tukey Test, significance was demonstrated for all four solutions, but the iodine 100% death concentration was found to be the most significant with a .465 value compared to a Dmin of .025. The research hypothesis, that if *Turbatrix aceti* were exposed to the smallest, still lethal concentration of liquid iodine or liquid chlorine, then the liquid iodine would be more effective, was supported. In the end, liquid iodine was found to be an inexpensive method of preventing roundworm infections as the average cost of liquid iodine is only 32 USD per kilogram, while the cost of a widely used waterborne roundworm treatment drug, Mebendazole, is 32 USD per gram.

Keywords: *Turbatrix aceti*-a species of infections waterborne roundworms that infest drinking supplies; Liquid Iodine-a solution of potassium iodide with iodine in water; Liquid Chlorine-a liquid solution created by the bubbling of chlorine gas; Mebendazole-an anthelmintic drug used to treat roundworm infections

Abbreviations: LD50-a hazardous agent concentration with enough potency to eliminate half of a trial group; LD100-a hazardous agent concentration with enough potency to eliminate all of a trial group

Introduction

Worldwide, roundworm infections have been a prevalent issue, as they impacted nearly one billion people worldwide in 2020 [1]. Although these infections have been easily prevented in first world countries, they have remained a large issue in impoverished nations. In Central America, waterborne roundworm infections have been especially prevalent due to the humid climate and relative lack of filtration infrastructure. Prior research has indicated that when exposed to inexpensive agents such as liquid chlorine, waterborne nematodes, such as Turbatrix aceti, can be killed. Unfortunately, once these roundworms have been ingested by someone drinking from an unpurified water source, they can proliferate by releasing eggs into the intestinal tract of their host [2]. Following infection, roundworms such as *Turbatrix aceti* interact with the host's microbiota by producing antibacterial material to survive, causing nausea and sickness for the host [3]. Research has also indicated that inexpensive water purification methods, such as iodine, can eliminate nematodes, like Turbatrix aceti, as well [4]. This species provided an excellent model for my research, as they cannot infect humans, but are still waterborne. In response to increased rates of infection in Central America, the following research question was developed- "What effect will potable quantities of liquid iodine and liquid chlorine have on *Turbatrix aceti* roundworm survivability?"

Based on this question, the purpose of this study became directed at evaluating the feasibility of liquid iodine and liquid chlorine as cost effective methods of prevention for water borne roundworm infections. These infections are prevalent, especially in areas with humid climates and a lack of infrastructure. It was vital that these methods be investigated to provide research backing a viable solution to a human health issue in many parts of the world. Currently, the prevailing treatment method for roundworm infections is a drug called Mebendazole, which costs thirty dollars per kilogram, and is entirely too expensive for Central American nations to purchase/distribute [5]. This meant that to find a water purification method that would be applicable to the people of Central America, the chemical agent would have to be not only biologically safe, but economically inexpensive as well. Liquid iodine fit this bill well, as it is already used to remove eighty chemical infectants in the United States and costs only thirty dollars for an entire kilogram [5]. All in all, this study was focused on finding a viable way to combat roundworms, which was highly related to the one billion people suffering from roundworm infections worldwide.

The main concepts that had to be understood for this research project were largely related to the infectious properties of *Turbatrix aceti* and how these properties can be combated. Additionally, it was vital to understand how this species served as a model for more infectious ones. It also had to be understood that the *Turbatrix aceti* roundworms had to be cultured in apple cider vinegar and given apple slices for nourishment. The life cycle of *Turbatrix aceti* roundworms live ten months on average and birth forty-five babies every ten days [2]. Understanding this lifecycle along with how the infectious properties of *Turbatrix aceti* can be combated through the use of agents such as liquid iodine and liquid chlorine was vital for this project.

Roundworms have unique anatomy that allow them to live in a variety of conditions. *Turbatrix aceti* are a type of nematode that naturally inhabit warm bodies of water which are free of chlorinated agents. It is thanks to their unique bodily properties that these environments are ideal for *Turbatrix aceti* worms. These roundworms possess the ability to coordinate movements with surrounding roundworms to navigate through liquids more efficiently and locate food sources [6]. Their natural habitats can be replicated using apple cider vinegar which both provides a liquid medium and food source, as the roundworms can feed on the microbes used to create vinegar. *Turbatrix aceti* are able to survive, however, in various liquid environments, including drinking water [7]. In nations all over the world, these parasitic nematodes have infected drinking water supplies [8]. This ability of *Turbatrix aceti* to inhabit a variety of environments, including drinking water, makes them a threat to people living in warm areas without proper infrastructure to prevent the infectious properties of these roundworms [9]. Water purification agents such as liquid chlorine and liquid iodine have been proven to combat nematode proliferation. Waterborne roundworms are extremely prevalent in humid areas, especially those that have limited treatment options. Despite this, there are accessible methods of preventing the contraction of roundworm infections altogether [4]. Among these is the use of commonplace commercial disinfectant agents, such as 70% ethanol, which has been shown to eliminate up to 99% of the roundworms and eggs exposed to it [10]. Although these solutions are accessible, they are not safe to ingest or to purify water supplies with. To eliminate water borne roundworms and their offspring, other accessible and cost effective purification methods must be used.

Iodine, a naturally occurring element, is commonly used in the purification of water supplies. These cleansing properties apply to water borne roundworms as well, as liquid iodine has been shown to eliminate over ninety percent of roundworms and eggs in water supplies [4]. Better yet, these drastic results were achieved using potable quantities, meaning that liquid iodine can be used to eliminate water borne roundworms without contaminating water supplies. Another commonly used cleaning agent, liquid chlorine, has also been found to eliminate harmful agents in water supplies, including *Turbatrix aceti* [1]. Similarly, to liquid iodine, liquid chlorine has been shown to have this impact even in potable levels of just four milliliters per liter. Additionally, water sanitation using chemical agents like these was found to be highly effective in reducing the prevalence of roundworm infections in Togo, a humid nation lacking adequate infrastructure [11]. It is clear that disinfectants can be highly effective in the regulation and prevention of roundworm infections, especially in places where cost-effective infection prevention methods are most needed.

Agents used to eliminate roundworms from water supplies, such as liquid iodine and liquid chlorine, function by inducing irregular water circulation through the cell membranes and destroying organ tissues in water borne roundworms [12]. Ultimately, liquid iodine and liquid chlorine provide a cost-effective way to prevent water borne roundworm infections. This can truly be seen in the fact that liquid iodine costs only thirty-two dollars a kilogram while more regular treatment methods for roundworm infections, like Mebendazole, cost nearly thirty-two dollars per gram alone [5]. This shows that iodine is far less costly than regularly used treatment methods, and offers a prevention method.

Based on this research question, I hypothesized that if *Turbatrix aceti* were exposed to the smallest, still potable concentrations of liquid iodine or liquid chlorine, then the liquid iodine would be more effective. Since several variables were manipulated in this study and the *Turbatrix aceti* were randomly assigned to groups, this was a true experimental design. In line with this principle, the independent variables were the types of chemicals used to treat the nematode infested water while the dependent variable was the life expectancy of the roundworms, or their percent survival rate. There were also controlled variables present in this research project, like how the *Turbatrix aceti* roundworms were cultured in the same amount of apple cider vinegar, were kept in the same type of jar, were held in the same light/temperature conditions.

Materials and Methods

Nematode infections, particularly those caused by waterborne species, such as Turbatrix aceti, impact nearly one billion people worldwide through their infection of water supplies (Else et al., 2020) [9]. In order to combat this issue using inexpensive liquid agents, it was vital to establish proper lab techniques and to find optimal concentrations of each solution. To complete my testing, it was essential to first establish guidelines for my technical proceedings in the lab. To practice these techniques, I conducted pretesting. First, I sorted the Turbatrix aceti, from Flinn Scientific, into tall specimen jars, containing 50 mL of unpasteurized apple cider vinegar kept at roughly 70 degrees Fahrenheit. I then placed two apple slices thin enough to see through into each of the two specimen jars I utilized. Next, to establish techniques for the lab I began extracting 10 roundworms in each trial run with a graduated pipette and placing them into testing vats. This allowed me to refine my roundworm extraction technique. I was then able to practice extracting the correct levels of liquid iodine and liquid chlorine using a micropipette.

From there, roughly 10 roundworms per trial were placed into testing vats using a scaling pipette. To accomplish the goal of finding liquid concentrations of both liquid iodine and liquid chlorine that would eliminate 50% and 100% of the roundworms exposed to them, I created solutions of varying percentages of liquid iodine mixed with apple cider vinegar. This was accomplished using small beakers for measurement wherein 90 uL of apple cider vinegar was poured and measured using hash marks on the side and a micropipette was used to extract the proper amount of Lugol's solution (liquid iodine). Then, this Lugol's solution would be added to the solution which was then resuspended before being diluted with water to create new concentrations for experimentation. For each concentration this process slightly varied. To elaborate, for the LD100 concentration of liquid iodine, 10uL of Lugol's solution was added to the tube to be followed by 90uL of apple cider vinegar. Then, 1400uL of water were added, leading to a .0667% concentration of liquid iodine. This also simulated a small percentage of a natural environment being microbes (like apple cider vinegar).

After waiting for 5 minutes for each solution to properly mix, I would place a microliter of the solution into each testing vat using a standard pipette and would then count the number of dead roundworms over 5 minutes under a microscope. Each solution was tested once to evaluate its validity and then those that showed promise were tested on 10 groups of roundworms for data collection. This pretesting process took two weeks at the start of the project and was largely characterized by varying levels of liquid iodine and liquid chlorine being extracted from their host vessels before being mixed with water in smaller beakers. Thankfully, the same solution formation process could be utilized for each liquid substance.

Then, to test the impact of liquid chlorine in potable levels on the survivability of *Turbatrix aceti*, I replicated the aforementioned process by extracting roughly 10 roundworms per trial using the scaled pipette. They were then placed into the testing vats so that they would be properly contained while solutions were being made. I then utilized small beakers to pour 1mL of water into the container to be followed by varying levels of liquid chlorine combinations. Then, each testing vat was met with a microliter of these solutions to evaluate effectiveness. After each trial, I would observe the roundworms under a microscope for a 5-minute period and record the death rates.

To keep a sterile environment, lab safety protocols were followed, aseptic technique was maintained, and I cleaned each piece of equipment before and after use. To extrapolate, I wore a lab coat and gloves, along with goggles, each time I was working with the roundworms to prevent contamination. I also sprayed my work space with 70% ethanol solution before and after working with my roundworms. When the time came to dispose of the roundworms, my instructor and I eliminated any remaining *Turbatrix aceti* through the use of an autoclave session at 120 degrees Fahrenheit for 20 minutes. After each trial, I then disposed of any equipment that could not be sterilized as bio waste.

Following data collection, I had my data stored into an Excel spreadsheet, which allowed me to run a two-way analysis of variance (ANOVA) test, as my dependent variables and independent variables were measurements that each had multiple components. In other words, I had multiple independent variables and multiple dependent variables based on survival percentages of both 50% and 100% concentrations.

Results and Discussion

Statistical results

The results of this study, examining the impact of liquid iodine and liquid chlorine on the survivability of *Turbatrix aceti* nematodes, found there to be a significant difference between both experimental groups and the control group. I worked to find two solutions for both liquid iodine and liquid chlorine- one that would eliminate all roundworms and one that would eliminate half, serving as an LD50. This was completed by mixing varying levels of liquid iodine and liquid chlorine with water to create five milliliters total of each solution subset. Initially, the solutions were composed of 50% iodine or chlorine and were tested on groups of ten Turbatrix aceti. Following many trials at decreasing thresholds of liquid iodine and liquid chlorine, I arrived at the optimal levels of each solution agent. This resulted in the discovery that .0667% of a solution would need to be liquid iodine to kill the *Turbatrix aceti* nematodes with a 100% efficiency rate, while the other solution had to be .20% liquid chlorine to work in the same manner. Similarly, solutions composed of .0333% liquid iodine and .10% liquid chlorine were developed to eliminate half of each *Turbatrix aceti* trial group. To find these concentrations, I isolated groups of roughly ten *Turbatrix aceti* worms from their culturing vessels and placed them into testing vats. I then administered one microliter of the experimental solutions using a micropipette into each vat.

Following each treatment administration, I waited five minutes and counted each trial group's surviving *Turbatrix aceti* using a microscope to evaluate the solution's effectiveness. There was a significant difference within the data overall as the reported p-value reported by the Excel analysis was $3.96 * 10^{-20}$, which was far below the set alpha value of .05. As there were multiple independent variables, I conducted a post-hoc Tukey Test to determine where the significance between groups was. This revealed that there was significance for all four solution groups, as every group boasted a reported value above the Dmin of .025. The LD50 concentrations for liquid iodine and liquid chlorine held reported values of .455. Despite this reported value being the same, the liquid iodine group did achieve this level of significance with a solution composed of only .0333%, while a solution composed of 10% liquid chlorine was required to achieve these results. Both the 100% death rate liquid iodine and liquid chlorine groups also held the same level of reported significance, as their Tukey Test values were both .465. However, liquid iodine proved to be more effective overall as this level of significance was achieved with a lower solution concentration (see Table 1).

Table 1: Average Death Rate By Percent of Hazardous Agent Concentration.

Concentration	.0667% Iodine	.0333% Iodine	.20% Chlorine	.10% Chlorine
Death Rate	100%	55%	100%	54%

Interpretations of results

Overall, I rejected the null hypothesis, that there would be no significant difference between each group's survivability, based on the low p-value I attained and the indicated significance of the liquid iodine groups, which led the alternate hypothesis to be accepted. This alternate hypothesis stated that there would be a significant difference between group survivability. I also concluded that my initial hypothesis was supported which stated that if *Turbatrix aceti* were exposed to the smallest, still potable concentration of liquid iodine or liquid chlorine, then the liquid iodine would be more effective. This was shown to be true, as liquid iodine only costs roughly thirty dollars per kilogram, which is vastly more affordable than Mebendazole at thirty dollars per gram.

My initial hypothesis stated that if *Turbatrix aceti* were exposed to the smallest, still potable concentration of liquid iodine or liquid chlorine, then the liquid iodine would be more effective. This was shown to be true, as liquid iodine only costs roughly thirty dollars per kilogram, which is vastly more affordable than Mebendazole at thirty dollars per gram. Additionally, although .0667% is a relatively high level of iodine overall in terms of concentration, this is a drinkable level as in extreme cases, up to 10% iodine solutions have been used to cleanse water supplies while more streamlined relief methods were on hold, while roughly .007% of water being composed of water can be an accepted level in unstable regions [13]. Liquid chlorine was not found to be effective in the slightest, as both twenty percent and ten percent are both too high a level to be consumed on a large scale. In the end, liquid iodine was shown to be an effective, cost efficient method of eliminating water born roundworm species such as *Turbatrix aceti*.

Throughout this experiment, none of the data was found to be extreme as each solution with an intended death rate of 100% met that threshold for each trial group. However, the percentage of liquid chlorine required in those concentrations was unexpected, due to the 100% death rate solution having to be composed of 20% liquid chlorine, which was much higher than prior research had indicated would be necessary. This could have been due to the grade of liquid chlorine used by the alternate research study. Prior to beginning the study, it was essential to refine the ability to extract the *Turbatrix* *aceti* in groups of roughly ten from their culture vessels and place them into trial vats using a pipette so that the experimental solutions could be applied to them using a micropipette. Despite this, there were not any major problems that occurred during the research study itself. However, prior to the start of the research study, it was difficult to find a proper solution concentration to start at. In other words, as the study was based around finding the lowest level of liquid iodine and liquid chlorine that could be used to eliminate both 100% and 50% of the roundworms in each trial group, the largest issue was deciding what levels to test first. Initially, a solution containing 50% of each purification agent was applied to the *Turbatrix aceti* trial groups, which was vastly over the amounts that ended up being used in the final solution applications. Regarding limitations of the study design, one minor issue could have been present in the exact amounts of each intended agent not being placed into the solutions. In other words, it is possible that over the course of the study, small variations were present in the intended levels of liquid iodine and liquid chlorine and the actual levels taken from the supply containers with the pipettes due to human error. To fix this, one could refine their pipette technique to a near perfect level prior through experimentation or by using more finite micropipettes.

Future outlook

Following the analysis of this data, it was determined that impoverished communities could feasibly use liquid iodine as a water purification agent to eliminate water borne roundworm species from local supplies. It was hypothesized that if *Turbatrix aceti* roundworms were exposed to liquid iodine and liquid chlorine, then the liquid iodine would be more effective in eliminating them. This was supported by the data, as the liquid iodine solution was able to eliminate 100% of the roundworms exposed to a solution with a concentration of only .00625% liquid iodine. This result was anticipated based on prior research, as iodine was found to drastically reduce *Pseudocapillaria tomentosa* survivability to below 5% in their zebrafish host sites after just two hours of drying following treatment application [4]. Based on its use in aquatic environments, it was predicted that the liquid iodine solution would be highly effective in eliminating water borne roundworms. Additionally, liquid chlorine has been demonstrated to eliminate harmful bacteria and organisms in water supplies with levels only up to 4 milligrams per liter [1]. My study was in relative conflict with these findings, as the liquid chlorine solutions were not found to be as effective in eliminating *Turbatrix aceti* trial groups. The 100% elimination rate liquid chlorine solution had to be composed of 20% liquid chlorine to achieve a complete death rate for each trial group while the chlorinated LD50 solution required 10% chlorine. In other words, my research did not find liquid chlorine to be as effective as other researchers [1].

As this study did show a cost effective, viable method of eliminating water borne roundworms in the form of liquid iodine, future research could be based around a larger scale testing of this agent. This could, for example, include more trial groups to further verify effectiveness of the solution. Or, further experimentation could include testing liquid iodine in combination with other purification agents to see if a lower total concentration could be found. Lastly, alternate species could be used in a similar study to examine the effectiveness of liquid iodine or liquid chlorine with other organisms. Although this study does allow for expansion, it is important to recognize that in its present state, it still showed a viable option for reducing the level of roundworm infections in humid, impoverished areas. If water sources could be more effectively purified in such areas, then the overall prevalence of roundworm infections would fall drastically, as the risk of water supplies would be almost none following treatment. This I supported by the fact that in Togo, government officials were able to eliminate a variety of different infectious roundworms from their water supplies using a mixture of solutions including iodine [11]. Overall, liquid iodine was shown to be a viable and affordable way to combat water borne roundworm infections.

The purpose of this research study was to evaluate the effectiveness of cost effective agents, such as liquid iodine and liquid chlorine, in eliminating water borne roundworms such as *Turbatrix aceti*. As water borne roundworm infections are particularly prevalent in impoverished countries with humid conditions, it was imperative to find a viable method of reducing the threshold of these infections. Ultimately, this study achieved this goal, as liquid iodine was found to eliminate roundworms in *consumable levels*, all while costing 1/1000 of what Mebendazole does. This means that overall, my research identified a water purification method that will eliminate the *prospect* of infections all while operating at 1/1000 the cost of the current treatment method, which entails forcing developing nations to pay one thousand dollars per gram of Mebendazole after infections have already been contracted. Ultimately, this study successfully identified a method of water purification that developing nations can purchase for a tenth of a penny to each dollar the current method costs.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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