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Destruction of *Giardia Lamblia* by electrical treatment of infected tab water

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Protozoan parasites are responsible for the majority of gastrointestinal infections and even drinking treated tap water was reported risk factor for sporadic giardiasis. Variety of technologies has been used for elimination of *Giardia* cysts as well as removing other microbial contaminants and particles. The cysts are usually identified by their indistinguishable morphology. We applied direct current (DC) electrical charges for 1 to 4 min, 10 mA or 12 V, and 15 mA or 18 V in a handmade electrolytic cell filled with a prepared 10^5 cysts per ml emulsion, to examine its effectiveness as cysticide. Fresh human stools with *Giardia*, stained with Eosin Gelblich, were used to prepare wet mounts. Samples were examined by conventional light microscopy for the presence of cysts. Temperature and pH of the emulsions were measured in all stages. DC electrical charges equal or more than 15 mA (18 V in our circuit), in 2 min are practically lethal for *Giardia* cysts found in human stools, in a watery base with this concentration. pH of the emulsions varied between 3.5 to 8.8 in 22-24.4°C, when the currents were applied. 5 min after closing the DC electrical circuit, the tank water was treated and could be used.

Key words: *Giardia lamblia*, water treatment, direct electrical currents, cysticides.

INTRODUCTION

Waterborne giardiasis has been reported in epidemic form among humans in many countries and drinking water is the most reported vehicle of spread. Swallowing water while swimming, eating lettuce and even drinking treated tap water are reported risk factors for sporadic giardiasis in the United Kingdom (Stuart et al., 2003). Intestinal protozoan parasites are responsible for the majority of gastrointestinal infections in subtropical areas (Ravdin, 1995) and about 58 million children suffer from giardiasis every year (Escobedo et al., 2009). Although higher incidence of intestinal parasites in lower socio-economic societies has been reported as a health inequities but spreading of giardiasis caused by drinking water is also reported in several states of United State (Espelage et al., 2010).

Environmental occurrence of *Giardia* has been reported in variety of sources. Jakubowski and his colleagues examined raw sewage waste water in 11 cities of Unites State and reported a correlation between raw sewage cyst level and reported cases of giardiasis (Jakubowski et al., 1991). Mayer and Palmer (1996) examined samples from a large metropolitan wastewater plant in California. They reported 13000 cyst/L in influent and 11 cyst/L in secondary effluent samples.

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Cysts have also been found in surface water, drinking water, groundwaters, cisterns, soil, surfaces and foods in North America and United State (Hancock et al., 1997; Crabtree et al., 1996; Cornell University Report, 1993; Cody et al., 1994 and Fayer et al., 1998). Viable *giardia* cysts are highly infective for humans. In a controlled clinical study of volunteers who were fed *giardia* cysts contained in conventional gelatin capsules, eight dosage levels ranging from 1 to 1,000,000 cysts per capsule have been examined. A single dosage of ten cysts were found to be clinically infectious in human (Rendtorff, 1979).

Results of the latest (2005) National Research Project in Iran showed that the prevalence of gastrointestinal parasitic infections has been about 19.3% while 10.9% of the patients suffered from *Giardia Lamblia* (Sayyari et al., 2005). Lack of water filtration as well as inadequate personal hygiene, socio-economic status, poverty, lack of sanitation and geographic factors were mentioned as the main reasons causing parasitic infections in Tehran, the capital of Iran (Shojaei Arani. 2008).

Giardia genuses are binucleate, flagellated protozoan parasites which attach to the wall of the human small intestine. *G. duodenalis*, *G. Intestinalis* or *G. lamblia* are common variety of symptoms in human Giardiasis. *G.lamblia* pyriform bodies range from 9 to 21 µm wide and 2 to 4 µm thick. They are identified by their two morphologically indistinguishable anterior nuclei, eight flagella, two central axonemes, microtubular median bodies and a ventral adhesive disk (US. EPA report, 1999). *Giardia* could be transmitted via the fecal-oral route of exposure. Giardiasis can be an asymptomatic infection in humans but it may cause acute or chronic diarrhea, steatorrhea, abdominal cramps, bloating, flatulence, weight loss and vomiting (Hall. 1994).

Because of the resistance of *Giardia* cysts to environmental conditions, high prevalence and low infectious dose in humans, variety of water treatment techniques have been used to remove or inactivate *Giardia*.

Most frequent physically removal technologies to reduce water turbidity by removing microbial contaminants and particles are: conventional/direct/membranes such as microfiltration, ultra filtration, nanofiltration, and reverse osmosis/ slow sand/ diatomaceous earth (DE) filtration methods. They almost remove 99.9% of *Giardia* cysts when operated under appropriate coagulation conditions (US. EPA report, 1999). However, Ongerth (1990) found poor removal of *Giardia* cysts as the major deficiencies in the operation of three small water plants with either conventional filtration, direct filtration, or DE filtration. Because possible outbreaks occurred in filtered water systems, frequent sampling and monitoring of treatment effectiveness are necessary for effective removal of cysts. Inactivation of cysts up to 99% or more can be achieved by disinfectants although several factors including water temperature, pH, residual and applied disinfectant concentration, contact time; some particles which may shield cysts from contact may increase disinfectant demand. *Giardia* can be resistant to low dose of chlorine and chloramines and inactivation efficiencies of the various disinfectants are different. Jakubowski (1990) studied utilized water disinfectants effectiveness of inactivation of the cysts and sorted them in the following decreasing order: ozone, mixed oxidants, chlorine dioxide, iodine, free chlorine, and chloramines.

Slavik (1993) used a low-current, low-voltage electrical treatment as a means to either kill *Giardia lamblia* or prevent excystation of this protozoan.

Karanis et al. (1998) examined sensitivity of protozoan to Ultraviolet (UV) irradiation and found that reduction of *Giardia* cysts requires a dose of 1800 J/m2. The comercial UV units irradiate much less doses and are not reliable. The new generation of powerful UV devices showed capabilities to reduce to less than 90% of *Giardia* cysts at the maximum dose tested.

MATERIALS AND METHODS

In an experimental study, with a pilot scale study design with four constant repetitions, we examined the effectiveness of electrical direct currents (DC) as a cysticide for control of *Giardia* where treated tab water is not available. Fresh stools were used to prepare wet mounts that were examined by conventional light microscopy for the presence of cysts (Figure 2). Samples counted positive for *Giardia* based on their distinctive morphology.

Tab water was boiled for 30 min and kept in a sterile reservoir and cooled down to room temperature (23-25°C). Its pH was controlled before and after boiling. Four samples were collected in the University Reference Lab from the Giardiasis patients diagnosed by finding *G. lamblia* and cysts with their feces. In each case, 1 ml of the fresh stool sample was added to the boiled water to prepare diluted emulsion. The emulsions then passed through 2 layers of sterile filter pads to clear up and centrifuged at 900 *g* for 1 min. The sedimented layers were diluted again to reduce sample turbidity. The samples were monitored by staining with Eosin Gelblich dye solution for detection of cysts. 0.1 ml of sample was mixed to 1 ml of prepared dye and stained cysts were mounted on hemocytometer counting chamber of the Light microscope using an oxford sampler pipette. Based on this counting method, 4 containers of the emulsion with 10^5 cysts per ml was prepared. Temperature and pH of the emulsions were controlled before electrical interventions. 10 ml of controlled emulsion were decanted by a pipette in a handmade, 10 CC, semi-cylindrical electrolytic cell which had two platinum plates (electrodes) at the two ends of the main slot (Figure 1).

Duration of the DC electrical treatments were 1 to 4 min and two low currents of 10 mA or 12 V, and 15 mA or 18 V (in our electrolytic cell), were applied at each of the four durations. Temperature and pH of the emulsions were measured inside the tank, soon after each particular dosage of currents applied.

In the next step, the whole content of the tank was poured in the test tube for further samplings. Samples were also taken from the electrode's surface by swab sticks. After similar staining, all samples were mounted on hemocytometer counting chamber to be exactly monitored by the same rater. The same process was repeated 96 times in different durations, intensities applied to the four stool samples and three times repetition.

Electrolytic cell was washed and carefully cleaned using alcohol, sterile water and cotton at each repetition. The wall of the tank and the surface of both electrodes were checked after each washing by

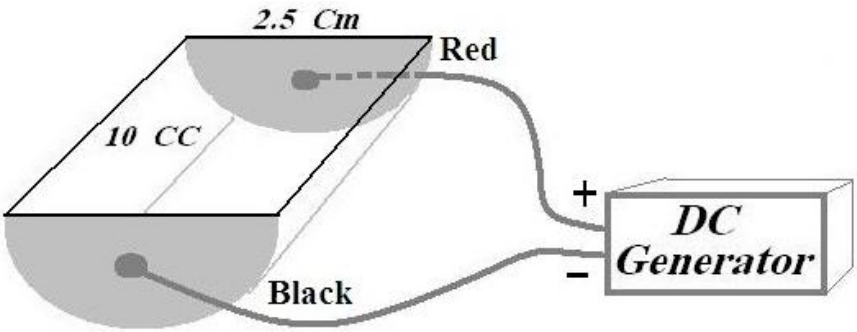

Figure 1. The electrolytic cell used to apply DC electrical charges; handmade by the means of two semi-circular Platinum plates and a semi-cylindrical tube.

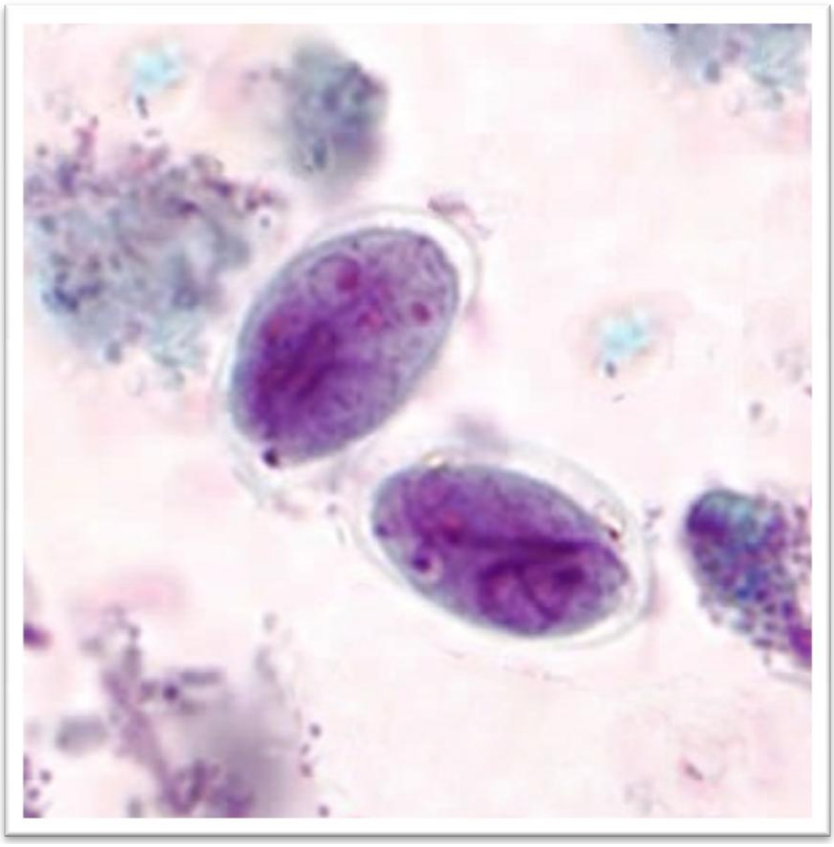

Figure 2. *Giardia lamblia* cysts stained with Eosin Gelblich.

three separate wet swab samplings.

We used a programmable electrical current generator to apply the electrical currents with pre-set parameters (3646A – DC power supply, Circuit Specialists Inc. USA). The conventional hand controlled generators could not be used for such short time current durations with the constant level of intensity since there is no distinction between impedance and resistance in DC electrical circuits. To calculate the exact current intensities at different sites of electrolytic cell, we measured the Ohmic resistance of each part of the tank by an impedance meter (ZM-104A, TOA, Japan). In each set of samples and before opening the electrical current, electrical resistances of the four following parts of the circuit were measured: 1) The whole circuit [from the tip of Anode wire [+] to the tip of Cathode wire (-), Red wire to Black wire tips]; 2) Inside the filled tank (from mid-point of each wall, inside the emulsion); 3) Cathode resistance [from cathodic platinum plate (black side) to the middle of the tank]; anodic resistance [from anodic platinum plate (red side) to the middle of the tank].

Having the electrical resistances, the intensity of the currents were calculated based on the Ohm law; V = R x I, where V is the voltage of DC current on the generator device (volts), R is measured resistance (ohms) and I is the intensity of current (Amperes).

Table 1. Effects of direct electrical currents in various intensities and durations on concentration of the 10^5 cyst/ml emulsions.

Current	Time (min)				
	0	1	2	3	4
(mA)-(V)	**Cyst Concentration** - per ml				
10-12	10^5	10^5	8 x 10^4	5 x 10^4	2 x10^4
15-18	10^5	10^4	0	0	0

Table 2. Effects of direct electrical currents in various intensities and durations on the pH and the temperature inside the tank. When the electric current is open,variable pH of electrode sides noticed.

Time (min)	Applied currents (mA)			
	10	15	10	15
	Temperature (°C)		pH	
0	22	22	7.2	7.2
1	22.5	23	4.5 – 8.2	3.7 – 8.7
2	22.8	24	4 – 8.3	3.5 – 8.8
3	23	24.4	3.9 – 8.5	3.4 – 8.8
4	24	24.4	3.7 – 8.7	3.2 – 8.8

RESULTS

As the main finding of the study, *Giardia* cysts found in human stools, in a watery base with the concentration of 10^5 cysts/ml could be practically counted up to 0 whenever DC currents equal or more than 15 mA (18 V in our circuit) are applied for 2 min (Table 1).

The measured electrical resistances were: 0.5 KΩ close to the anode electrode, 0.4 KΩ close to the cathode electrode, 1 KΩ inside the tank, and 1.2 KΩ for the whole circuit. Therefore, for the 18 V current, the current intensities which caused the complete cysticide effects should be: 36 mA close to the anode electrode, 45 mA close to the cathode electrode, 18 mA inside the tank, and 15 mA for the whole circuit.

Tap water pH did not change after 30 min boiling but the emulsions pH changed considerably during DC electrical treatments. When the currents were opened, pH reached to the minimum amount of 3.5 close to the anode and maximum amount of 8.8 around the cathode. These changes reversed to the normal rates after 285 s (less than 5 min).

When an electrical current passes through a circuit with resistance, heat production is inevitably expected. In our electrolytic cell, maximum temperature enhancement after 4 min of DC electrical treatment was 2.4°C (Table 2).

DISCUSSION

Although viability determination might not be necessary for determining the effectiveness of the treatment process for physically removed or injured cysts, but it is very important in assessing disinfectant efficacies of treated water before drinking.

We used dye staining, morphological criteria and cyst counting as the post treatment procedures to determine the efficacy of each particular electrical intervention.

The discontinuity in gradual relationship between electrical current duration at its proper intensity as a cysticide was expected, since the lethal charge level of electricity for *G. lamblia* should have a sharp threshold instead of variable broad ranges.

As a result of polarization of electrodes and formation of H^+ ions around cathode and OH^- ions around anode, pH of electrolytic cell was variable after closing DC currents. These changes are unstable in watery environments and usually reverse when the ions combine and turn to water again (http://en.wikipedia.org, 2013).

The parameters we used in the electrolysis of *Giardia* cysts should be effective on cysts from human. The host source of the cysts may play some undefined roles. Hibler and Metzger (1974). also suggested this possibility. However, this speculation will require several sets of experimentations on variety of *Giardia* cysts from different sources to be approved or ignored.

Viability of cysts could be significantly affected as results of pH changes. Rubin et al. reported an average viability of 91% at pH 5, 56% at pH 7, and 62% at pH 9 all at 15° C, with a variability not exceeding ±5% (Rubin, 1989). The viability of cysts in our preparations was matched and count controlled to have emulsion with 10^5 cysts per ml. The emulsions pH was changed from 3.5 to 8.8 in 22 to 24.4°C when the currents were applied (Table 2).

Although both recent parameters may have influenced

the viability of Cysts but other factors, such as electric DC charge, should be responsible for the Zero viability (all killed). A positive correlation could be seen between the rate of applied DC voltage, intensity, duration and its efficacy to massacre the cysts.

The duration of the low current, low voltage of DC electric charges were shown in this study to be cysticidal for *Giardia* at room temperature and are similar to those suggested by Slavik et al. (1993).

Slavik found that electrical treatment can kill *Giardia* trophozoite in 5 min or less in both static and flow through systems. Electric treatment was also able to prevent excystation of *G. lamblia*. Hass and Aturalive (1999) also studied the effects of very short duration pulses of high voltage electrical current (Electroporation) on the viability of *Giardia* cysts, Cryptosporidium oocysts, and reported a minor effect on the cyst survival when the electrical pulses were used. In electroporation, usually short term, several hundred volts of alternative electrical current would be applied on the cell plasma membrane in molecular biology to change its electrical conductivity and permeability. Haas and Aturaliye (1999) used it in the presence of free chlorine, combined chlorine, hydrogen peroxide and potassium permanganate. They concluded that the combination of electroporation and those chemical disinfectants could produce superior inactivation of cysts and would be the treatment of choice to inactivate resistant protozoa in water. In our application of DC electricity, the only possible biological effects could be the enhancement, reduction or block of cyst's membrane caused by the bipolar DC charge of electricity somehow similar to the Iontophoresis of the skin.

The technique, if used in water treatment plant, would be safe without adding extra chemicals to drinking water. In DC electrical circuits, the only hazard could be the electrical shock. The perception of electric shock can be different depending on the voltage, duration, current, path taken, frequency, etc. Current entering the hand has a threshold of perception of about 5 to 10 mA for DC. The shocks can be felt only in sudden touch or in opening-closing of the current (http://www.electricityforum.com. 2013). Therefore, the current intensities used in this method could be at sensory threshold of perception.

Since the findings of the study on effectiveness of DC electrical treatment is based on results of laboratory experiments, additional studies should be conducted to compare the effectiveness of electricity under representative conditions in natural waters and local water plants.

Our results clearly demonstrate that viable *Giardia* cysts can be destroyed reliably by a short time DC electrical power and that this destruction can take place even in a few minutes using a simple electrolysis tank. We should emphasize that when electrical treatment is used as a cysticide against *Giardia,* attention should be given to the pH, ions, change after DC treatment and the tank water should be used at least 5 min after closing the electrical circuit.

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