

## Research Paper

# Deactivation of *Ascaris suum* eggs using electroporation and sequential inactivation with chemical disinfection

C. Niven, C. B. Parker, S. D. Wolter, M. H. Dryzer, C. B. Arena, B. R. Stoner and E. Ngaboyamahina

### ABSTRACT

Electroporation has been evaluated as a potential backend wastewater treatment for deactivation of *Ascaris suum* eggs in buffer solution. Initial results indicate that eggshell permeability is affected by the pulse train electric field strength and duration. Coupling electroporation with chemical exposure, using low concentrations of commercially available disinfectants, allows oxidizing agents to pass through the complex strata of the *A. suum* eggshell, specifically reaching the innermost embryonic environment, which leads to successful deactivation compared to either method used separately. The aim of this work is to identify and develop an alternative technique that efficiently inactivates helminth eggs present in wastewater.

**Key words** | *Ascaris suum*, chemical disinfection, eggshell permeability, electroporation, helminth deactivation, wastewater sanitation

### HIGHLIGHTS

- Soil-transmitted helminthiasis (STH) is a significant global health threat that primarily affects individuals in developing countries lacking safe Water, Sanitation, and Hygiene (WaSH) management. Current methods for helminth inactivation, such as exposure to high temperature and pH or soaking in chemical agents, are successful though lack consistent inactivation rates across studies. Furthermore, these methods require capital expenditure which may restrain their implementation in resource-constrained regions.
- Electroporation is a technique known to permeabilize the eggshell of a non-parasitic helminth surrogate, *Caenorhabditis elegans* (*C. elegans*). This is accomplished by using pulsed electric fields to cause pore formation within the lipid bilayer of cell membranes; this technique was also found to permeabilize the innermost layer of the eggshell strata, the lipid layer. The creation of these pores is hypothesized to cause irreparable damage and increase the vulnerability of the embryo by allowing the passage of deleterious chemicals, such as chlorine, into the egg. Exposure to these changes may ultimately create an unfit environment for continued embryonic development thereby inactivating the eggs.

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- Though the eggshell of helminths is found to be more resilient than that of the non-parasitic surrogate, the permeabilization of eggshell strata, facilitated by electroporation, was found to be successful in decreasing *Ascaris suum* viability.
- For the first time, we demonstrate that electroporation coupled with sequential inactivation using chlorine can successfully inactivate *Ascaris suum*. By directly delivering the chlorine into the eggshell, we are minimizing the overall amount of time and energy input that is necessary for either electroporation to cause irreparable membrane damage or chemical exposure to dissolve the outer layers to successfully inactivate the helminth eggs.

## INTRODUCTION

The health of approximately 2.5 billion people is negatively impacted by the prevalence of polluted water due to inadequate sanitation facilities and the consequent inability for individuals to practice safe personal hygiene methods (Jiménez *et al.* 2010; Naidoo *et al.* 2016; Gyawali 2018). The global health issue associated with unpotable water sources suggests an increased risk of infectious disease not only caused by direct consumption or contact with wastewater but also for its use in 7% of the world's total irrigated land (Jiménez *et al.* 2010).

Soil-transmitted helminthiasis (STH) is a global health threat that affects 1 in 5 individuals, primarily in developing countries lacking safe Water, Sanitation, and Hygiene (WaSH) management (Jiménez 2007; Bandala *et al.* 2012; World Health Organization 2012, 2019; Naidoo *et al.* 2016; Midha *et al.* 2018; U.S. Census Bureau 2019). Ascariasis, caused by the parasitic roundworm *Ascaris lumbricoides*, results in STH infection in humans, impacting over 10% of the world's population (Brownell & Nelson 2006; Knopp *et al.* 2012; Naidoo *et al.* 2016; Global Health Metrics 2017; CDC 2018; World Health Organization 2019). This species of helminth is incredibly resilient, as it is able to survive outside of a host amidst adverse environmental conditions due to its complex eggshell structure (Gamble *et al.* 1995; Mei *et al.* 1997; Brownell & Nelson 2006; Moodley *et al.* 2008; Perry & Moens 2011; Cruz *et al.* 2012; Katakam *et al.* 2014; Naidoo *et al.* 2016; Oh *et al.* 2016; Jiménez *et al.* 2017). Ingestion of an infective egg through fecal-contaminated soil, crops, or water, will prompt the egg to undergo the hatching process that causes morphological and chemical changes within the eggshell structure. The most relevant of these changes is an increase in permeability due to a mechanical

(O'Connor 1951; Naidoo *et al.* 2016) or enzymatic breakdown of the innermost lipid layer (Clarke & Perry 1988; Gamble *et al.* 1995; Mei *et al.* 1997; World Health Organization 2012; Katakam *et al.* 2014; Schmitz *et al.* 2016; Jiménez *et al.* 2017; CDC 2018). Composed of glycolipids called ascarosides, the lipid layer is impenetrable to most chemicals in order to maintain a stable, internal embryonic environment (Gamble *et al.* 1995; Mei *et al.* 1997; Dalton *et al.* 2006; Perry & Moens 2011; Choe *et al.* 2012; Naidoo *et al.* 2017; Renahan & Hong 2017). Only upon maintaining ideal environmental conditions, indicated by external stimuli, does the lipid layer permit *A. lumbricoides* to undergo the process of host infection, permitting further embryonic development and eventual hatching (Clarke & Perry 1988; Gamble *et al.* 1995; Geng *et al.* 2002; Brownell & Nelson 2006; Perry & Moens 2011; Bandala *et al.* 2012; Naidoo *et al.* 2016). The lipid layer in the complex eggshell strata serves as the final larval protection. Consequently, damage to this layer at early stages of development may negatively impact the embryo, as it becomes more susceptible to environmental stress and harm (Wharton 1983; Perry & Moens 2011).

Current techniques used for helminth inactivation through affecting the ova strata include exposure to high temperature and pH, UV radiation, soaking in chemical agents (i.e., chlorination/oxidation), and anaerobic/aerobic digestion (Bandala *et al.* 2012; Maya *et al.* 2012; Oh *et al.* 2016; Gyawali 2018). Though these methods have proven successful, there is a lack of consistency regarding inactivation rates among the many studies on this topic (Gyawali 2018). Furthermore, techniques such as oxidation and chlorination are impractical as stand-alone methods of inactivation

because they require extended periods of chemical exposure, to ensure an exchange of electrons, which is dependent upon oxidant concentration (Alouni & Jemli 2001; Bandala *et al.* 2012; Naidoo *et al.* 2016). Ultimately, these methods of inactivation may require significant capital expenditure and specialized technologies, which limits their implementation in resource-constrained regions (Maya *et al.* 2012).

Another promising approach is electroporation. Electroporation utilizes short-pulsed electric fields to increase cell membrane permeability by forming transient hydrophilic pores in the lipid bilayer (Tarek 2005; Kotnik & Miklavčič 2006; Schoenbach *et al.* 2007; Böckmann *et al.* 2008; Neu & Neu 2009; Deipolyi *et al.* 2014; Rego *et al.* 2015; Rems & Miklavčič 2016).

Electroporation has proven successful in porating the lipid bilayer of cell walls ( $\approx 5$  nm thick), which supports the approach of imparting a similar molecular response on a larger scale within the lipid layer of helminth eggshells ( $\approx 100$  nm thick) (Dalton *et al.* 2006; Schoenbach *et al.* 2007; Kotnik *et al.* 2012). The aforementioned pores would effectively penetrate the eggshell strata, increasing permeability and allowing access the formerly inaccessible embryonic environment (Tarek 2005; Kotnik & Miklavčič 2006; Schoenbach *et al.* 2007; Böckmann *et al.* 2008; Neu & Neu 2009; Rego *et al.* 2015; Rems & Miklavčič 2016). The authors have recently demonstrated the electropermeabilization of the eggshell of *Caenorhabditis elegans*, a nonparasitic helminth surrogate (Dryzer *et al.* 2019).

The present research evaluates the efficacy of electroporation to prompt *Ascaris suum* deactivation by increasing eggshell permeability. Through penetration of the eggshell strata, the embryo's vulnerability to its external environment will be intensified due to significant structural damage or necrosis. Four hypotheses are thus investigated in regard to electroporation prompting pore formation in the eggshell strata by decreasing *A. suum* viability. These hypotheses include (i) prompting pore formation using electroporation, (ii) using short treatment durations with high pulsed electric fields, (iii) using longer treatment durations across mid-range electric fields, and (iv) implementing sequential inactivation with chemical exposure.

The literature suggests that eggs in the latter stages of development are more sensitive to external environmental

factors due to the thinning and expansion of the eggshell to accommodate the development of more complex internal structures (O'Connor 1951; Maya *et al.* 2012). Though degrading the layers of the eggshell strata may result in decreased viability, targeting the innermost environment, rather than attempting to degrade the eggshell, may be more harmful to the ovum. The permanence and degree of such harm is highly relevant because the structural integrity and functionality of the lipid layer are imperative to helminth survival and subsequent viability (Wharton 1983; Clarke & Perry 1988). By experimentally causing an irreparable disruption to the lipid layer, the likelihood of continued embryonic development is expected to be compromised. This hypothesis emphasized the use of electroporation as a promising, novel method of helminth inactivation because it targets the functionality of the lipid layer (Dryzer *et al.* 2019).

Following the research of Dryzer *et al.* (2019), this paper reports on the electropermeabilization of *A. suum* as the recommended helminth surrogate per the ISO 30500:2018 standard. The aim of this work is to both identify and develop an alternative technique that efficiently inactivates helminth eggs present in wastewater. *A. suum* was used because it is only infective to pigs and has both morphological and genetic similarities to *A. lumbricoides* (Nelson & Darby 2001; Bandala *et al.* 2012; Leles *et al.* 2012; Naidoo *et al.* 2016; Midha *et al.* 2018). This work was performed to confirm the successful electropermeabilization of helminth ova and to provide a better understanding of treatment parameters that decrease helminth viability and prompt subsequent deactivation.

## MATERIAL AND METHODS

### Preparation of *A. suum* for treatment with electroporation

*A. suum* eggs were obtained from Excelsior Sentinel (Ithaca, NY). According to the supplier, they were isolated and cleaned from feces of infected pigs. Approximately 100,000 eggs were suspended in a 15 mL conical tube containing a non-sterile solution of 0.1 N sulfuric acid (liquid stock solution) stored at 4 °C in a BSL-2 certified laboratory at Duke University. Samples were prepared using 100  $\mu$ L of

liquid stock solution and centrifuged at 3,000 rpm for 10 min. 40  $\mu$ L of each pellet, or the egg-containing sediment, were removed and pipetted into electroporation cuvette test cells fitted with two aluminum electrodes situated 0.4 cm apart and containing 1 mL of phosphate-buffered saline (PBS) (20 mS/cm) (Nelson & Darby 2001). Each cuvette was inserted into an electroporation safety stand that connects the cuvette electrodes to a BTX T820 Electro Square Porator, which was used to treat the samples with predetermined experimental parameters, such as time and number of pulses (see Process 3 in Figure 1(b), Equation (1), and Table S1 in the Supplementary Information) (Dryzer *et al.* 2019). The electric field within each cuvette is uniform; therefore, each pulsed electric field was calculated by dividing the pulse amplitude (applied potential) by the distance between electrodes (Dryzer *et al.* 2019).

$$\text{Pulsed electric field} = \frac{\text{pulse amplitude (V)}}{\text{distance between electrodes (cm)}} \quad (1)$$

### Treatment with chemical exposure (chlorine and hydrogen peroxide)

Samples that received both electroporation and chemical treatment were dispensed into sterile conical tubes that contained either 10 mg/L of free chlorine (supplied by dilution of an off-the-shelf bleach solution, 6% sodium hypochlorite, with 5.7% available chlorine) or hydrogen peroxide (supplied by an off-the-shelf hydrogen peroxide solution, 3% hydrogen peroxide, and 97% purified water). Serial dilutions of each chemical oxidant were freshly prepared immediately before experimentation to ensure accurate concentrations were tested. After 20 min of exposure, the samples were thoroughly washed by centrifugation with DI H<sub>2</sub>O to remove residual oxidants, and the precipitate was aspirated to the original  $\approx$ 1 mL volume (Nelson & Darby 2001) (see Process 4 in Figure 1(b)).

### Incubation of samples

Each treated and untreated sample was dispensed onto a sterile Petri dish, and approximately 1 mL of 0.1 N H<sub>2</sub>SO<sub>4</sub> was pipetted into either the cuvette or conical tube to

wash off any eggs that potentially remained on the interior surfaces. 10 mL of 0.1 N H<sub>2</sub>SO<sub>4</sub> was added to each Petri dish before they were gently swirled. The Petri dishes were covered with plastic Petri dish lids prior to incubation at 25–28 °C for 28 days. The samples were aerated, by gently swirling the base of the Petri dish without the lid, and more 0.1 N H<sub>2</sub>SO<sub>4</sub> was added when necessary to prevent desiccation due to evaporation (Nelson & Darby 2001).

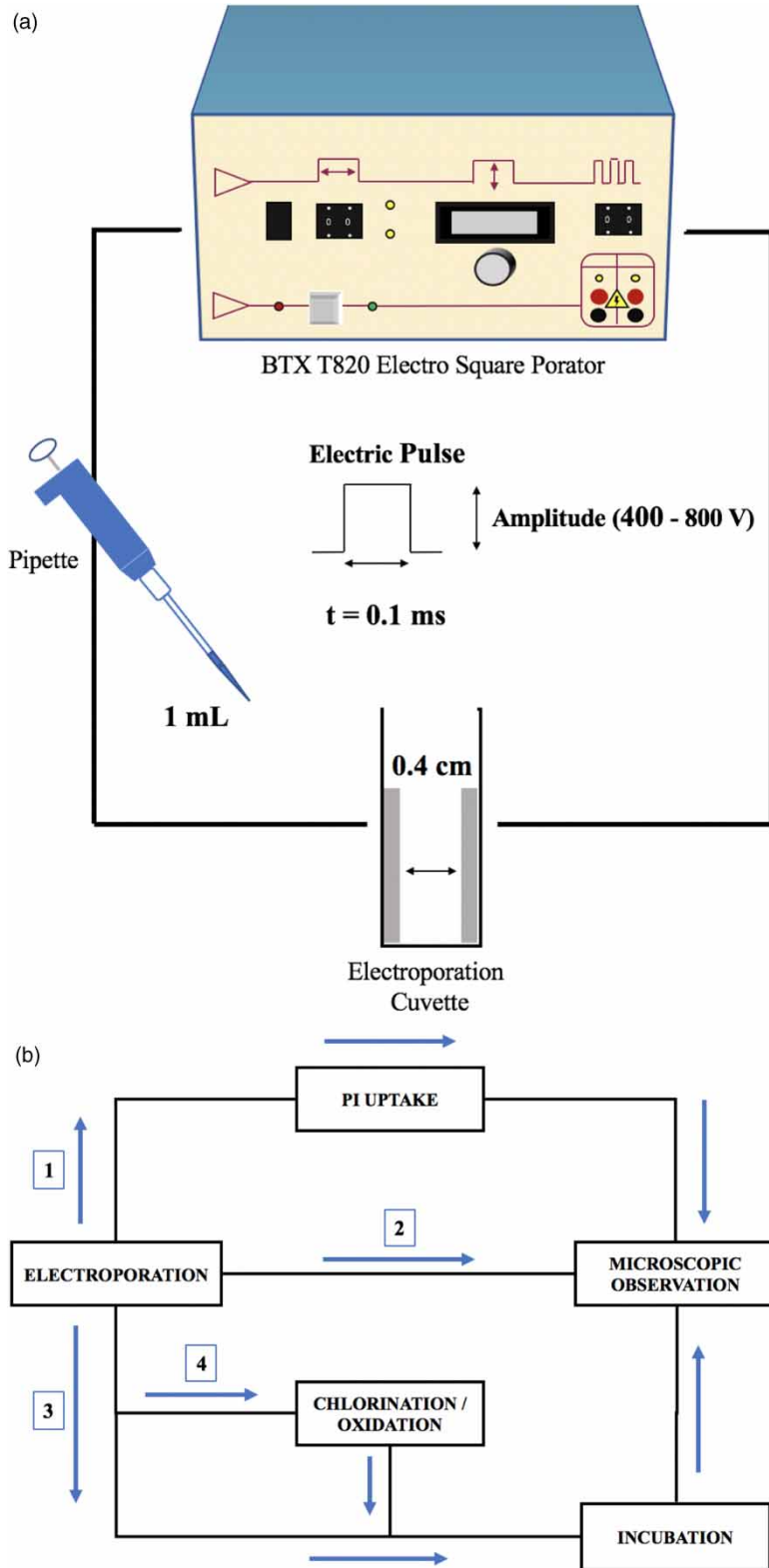
### Preparation of samples for observation

After 28 days of incubation, the samples were removed from the incubator, pipetted into 15-mL plastic conical tubes, and left overnight at room temperature to promote sample sedimentation. The conical tubes were then centrifuged at 3,000 rpm for 10 min before the supernatant was aspirated, resulting in a 3 mL sample. The conical tubes were centrifuged again, at 3,000 rpm for 10 min, and aspirated to 1 mL to obtain the final, egg-containing deposit.

An aliquot of each sample, approximately 20  $\mu$ L of the pellet-containing solution, was dispensed onto glass microscope slides. A glass coverslip was placed on top of each sample before the edges were sealed with clear nail polish to prevent leakage. The prepared slides were air dried and wiped with both saturated iodine and ethanol to prevent contamination prior to microscope observation. This process was also utilized for sample observation prior to incubation (see Process 2 in Figure 1(b)).

### Enumeration and assessment of egg viability

The *A. suum* eggs were enumerated and assessed, using direct microscopy observation using an EVOS<sup>®</sup> FL Auto fluorescent microscope, using the brightfield function, to classify viability. Viable eggs possessed developed larvae in the latter stages of development, and some of which were observed to be exiting eggs. The presence of these ‘hatched’ larvae was partly due to pressure from the glass microscope coverslip prompting the eggs to rupture; in fact, sealing a glass coverslip atop an incubated sample is a common method for *in vitro* hatching (Han *et al.* 2000; Nelson & Darby 2001; Kumagai *et al.* 2010; Karkashan *et al.* 2015; Naidoo *et al.* 2016). Though some burst eggs were observed without larvae, morphological indicators were used to



**Figure 1** | Schematics of (a) electroporation apparatus with sample setup and (b) experimental process.

determine viability, such as thinning of the interior eggshell and the presence of a larval sheath or cuticula (Geenen et al. 1999). If these indicators were not present, the egg was considered nonviable (O'Connor 1951; Cruz et al. 2012).

Post-incubation observation of *A. suum* is an accurate determination of viability because classification occurs after 28 days of incubation. This timeframe is in accordance with the United States Environmental Protection Agency (USEPA) incubation standard that requires 3–4 weeks of incubation, which considers the time needed to complete all stages of the *A. suum* life cycle (Cruz et al. 2012). Viability is thus based on the supposed potential for larval development.

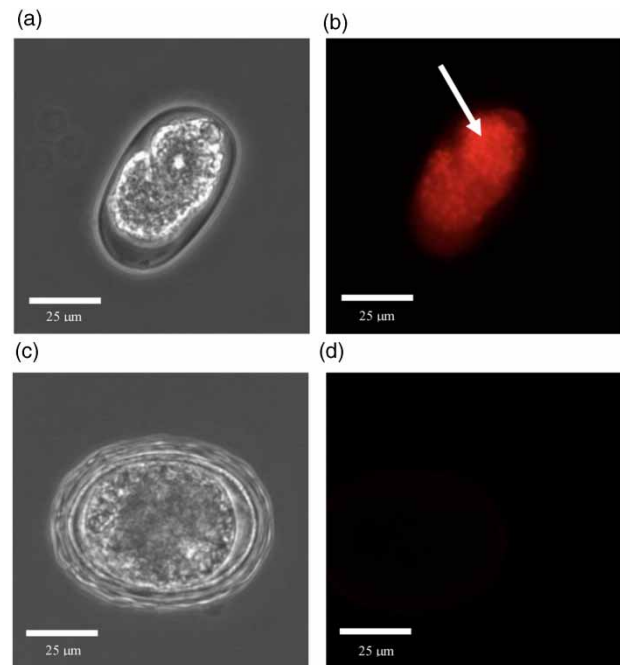
Percent viability was determined by counting the number of viable eggs, i.e., eggs possessing larval structures or larvae in latter stages of development, and the total number of eggs identified on each sample slide (see Equation (2)). Lower viability percentages indicate higher deactivation rates. Greater variability amongst percent viable observed for each treatment parameter is attributed to the smaller sample sizes. Any evaluation of viability that is not 0% is unacceptable, as some eggs remain active, and the treatment is deemed unsuccessful.

$$\text{Percent viability} = \frac{\text{No. of viable eggs}}{\text{total no. of eggs}} \times 100 \quad (2)$$

## RESULTS AND DISCUSSION

As previously reported (Niven et al. 2018), the use of propidium iodide, a fluorescent dye, was first evaluated as an indicator of pore formation. The evaluation of images of *A. suum* eggs, taken post-electroporation, illustrated internal fluorescence which indicated that the dye successfully penetrated the lipid layer (see Process 1 in Figure 1(b)). Internal fluorescence was observed across all tested voltage parameters. Therefore, electroporation successfully causes pore formation thereby permeabilizing the formerly impenetrable lipid layer (see Figure 2).

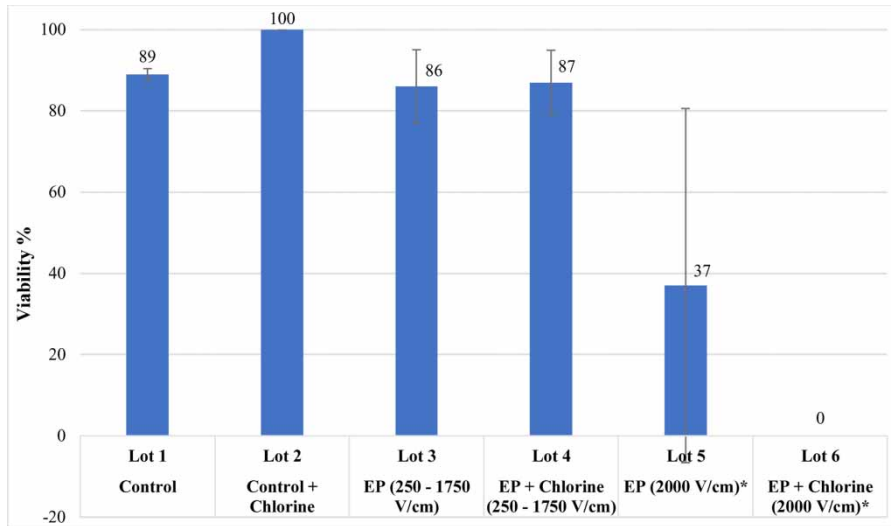
The examination of eggs that were treated with electroporation for less than 6 min, across various pulsed electric fields, suggests an insufficient amount of time to prompt substantial inactivation (see Lots 1–4 in Figure 3; Lots 3 and 4 treated with 2 and 4 min). The hypothesis that high electric



**Figure 2** | *A. suum* eggs observed under optical (left) and fluorescent (right) light. The comparison of *A. suum* egg treated with 1,500 V/cm for 2 min (a) and (b), against untreated *A. suum* egg control, (c) and (d). Fluorescence intensity observed in (b, white arrow) indicates eggshell permeabilization, whereas the dark area for untreated egg (d) indicates no dye uptake.

fields, for treatment durations shorter than 6 min, will exhibit an overall decrease in viability was rejected. Considering the survival of helminth ova depends on the resilience of their eggshell, it was then hypothesized that an extension of the treatment duration would allow deeper pores in the eggshell strata due to an increase in applied electric field prompting growth in the hydrophilic water channels penetrating the lipid membrane while flowing away from the center of the membrane (Tieleman et al. 2003; Winterhalter 2014). This would thereby explain a decrease in viability due to the membrane's inability to recover from increased mechanical stress. Results from these experiments indicated that 6 min of electroporation using three different pulsed electric fields led to lower viability percentages but did not lead to 100% inactivation of *A. suum* (see Lots 3 and 5 in Figure 3). It was concluded that electroporation, used alone in these experimental conditions, does not meet the requirements for a safe helminth treatment option, which supported the use of chemical co-exposure.

Due to the unsuccessful inactivation of all *A. suum* eggs, a modified approach to achieve deactivation was

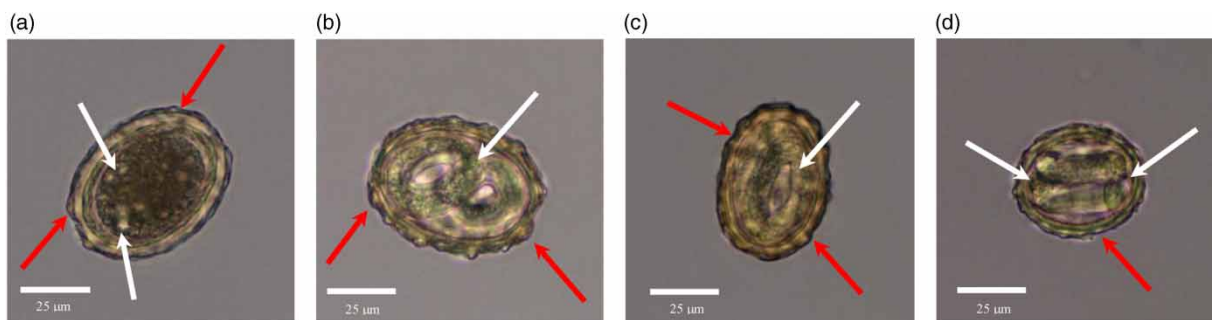


**Figure 3** | Differences in viability percentages for *A. suum* samples under varying treatment parameters observed post-incubation. All experimental data collected were used to calculate average viability percentages for each sample set. Standard deviation error bar for Lot 5 hypothesized to be impacted by potential differences in resistance to electroporation by eggs in varying apparent stages of development. \*Data combined for samples treated with 4–6 min of electroporation for viability calculations (see Tables S2 and S3 in the Supplementary Information).

implemented using proximal chemicals that are commonly used for wastewater treatment. It is hypothesized that irreversible electroporation occurs between 4 and 6 min with higher pulsed electric fields because the likelihood of poration causing irreparable damage, and subsequent cellular lysis, increases under extreme mechanical stress (Tarek 2005; Deipolyi *et al.* 2014; Rego *et al.* 2015). This is supported by our findings that electroporation with an electric field of 2,000 V/cm requires a treatment duration of 6 min to permit successful poration (see Lot 5 in Figure 3 and Table S2 in the Supplementary Information). Since viable eggs were still observed under these conditions, this finding further supported the use of chemical exposure as an alternative method of inactivation (Bandala *et al.* 2012; Naidoo *et al.*

2016; Oh *et al.* 2016) (see Table S3 in the Supplementary Information). It is to be noted that viable eggs, across all treatment parameters, typically possess an easily identifiable larval structure within, or exiting, the egg (see white arrows in Figure 4(b)–4(d)). Nonviable eggs are darker and have compromised eggshells and ‘bubbled’ interiors (see white arrows in Figure 4(a)) (Schmitz *et al.* 2016).

The identification of a reactive oxidant species (ROS) that can be generated in blackwater is of utmost importance. The solute composition of urine suggests that chlorine would be an effective target for electrochemical disinfection (Putnam 1971). Household bleach as well as other disinfectants have been evaluated for their effects on *A. suum* development; this research emphasizes the importance of



**Figure 4** | Examples of *A. suum* eggs observed post-incubation. (a) Nonviable and (b–d) viable (fully embryonated). White arrows in (a) point to globules that are a result of cell death and in (b–d) show the outline of the larval structure which indicates successful embryonic development. Red arrows in (a) through (d) point to the mottled outer layer. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/washdev.2020.192>.

chemical exposure. Inactivation using sodium hypochlorite, from household bleach, requires a minimum exposure time of 1 h in a high concentration of free chlorine, over 1,000 mg/L (Naidoo et al. 2016; Oh et al. 2016).

Research indicates that sodium hypochlorite is commonly used in *A. suum* sample preparation because it facilitates optimal microscopic observation by removing the outermost corticated albumin layer of the eggshell by degrading proteins and fatty acids present in the membrane (Smith 1991; Geenen et al. 1999; Nelson & Darby 2001; Maya et al. 2012; Naidoo et al. 2016; Harroff et al. 2017; Midha et al. 2018). To effectively dissolve this outer layer, *A. suum* eggs must be exposed to higher concentrations of sodium hypochlorite for at least 3 min (Smith 1991; Nelson & Darby 2001; Oh et al. 2016). Though decortication is said to increase the susceptibility of *A. suum* to treatment, the primary goal of this research is to use a low concentration of free chlorine, for a predetermined exposure time, to achieve chemical inactivation (Brownell & Nelson 2006; Bandala et al. 2012; Naidoo et al. 2016; Oh et al. 2016). In consideration of these findings, eggs were treated with a chlorine concentration of 10 mg/L. Both treated and untreated *A. suum* eggs, observed post-incubation, had mamillated layers of varying thicknesses, which indicated that the dilute bleach solution used in these experiments did not cause decortication (see outermost layers of eggs A and B, indicated by red arrows in Figure 4). Therefore, the solution cannot lead to *A. suum* inactivation.

Chemical exposure coupled with electroporation will give rise to a dose–response relationship with a ROS, such as free chlorine or oxygen (Dalton et al. 2006; Bandala et al. 2012; Naidoo et al. 2016, 2017; Oh et al. 2016). Delivery of ROS into the helminth eggshell, regardless of how deeply the pores penetrate the strata, would follow the same theory behind the effective use of EP (electroporation) for drug and gene delivery; therefore, it is hypothesized to decrease helminth viability (Neu & Neu 2009; Rems & Miklavčič 2016). The difference in influx of ROS will be impacted by the size of the pores formed considering larger pores contribute 86% of the fractional pore area, but smaller pores make up a greater percentage of the total pore area (Neu & Neu 2009). Ultimately, larger pores will take longer to seal, which may allow chemicals to pass more easily, and smaller pores may permit the passage of more fluid (Deipolyi et al.

2014). Both increase the amount of stress experienced by the membrane, the former being chemical and the latter being osmotic, therefore the significant contribution of both will be imperative to the overall destruction of helminths.

The aforementioned interpretation of pore depth was further tested by experiments evaluating pore transience after undergoing electroporation at 2,000 V/cm for 6 min. Samples that were exposed to 10 mg/L of free chlorine 10 s, 30 s, 1 min, and 2 min post-electroporation all led to 100% inactivation (see Table S5 in the Supplementary Information). This finding indicates that 2,000 V/cm for 6 min prompts the formation of pores that stay open long enough, meaning the cells cannot completely heal, thereby compromising the lipid membrane.

It was found that sequential inactivation using electroporation with 2,000 V/cm and exposure to 10 mg/L Cl<sub>2</sub> leads to 100% inactivation. Therefore, successful permeabilization of the *A. suum* eggshell requires a minimum electric field of 2,000 V/cm (see Lot 6 in Figure 3). Experimentation evaluating the use of another commercially available disinfectant, 3% hydrogen peroxide, for use in sequential inactivation, produced results that aligned with this finding (see Table S4 in the Supplementary Information). Samples treated with 1,750 V/cm for 6 min consistently have high viability rates, which could be the result of rapid pore sealing or insufficient pore formation. Therefore, electroporation at 2,000 V/cm prompts successful inactivation, regardless of the type of disinfectant. Ultimately, it is hypothesized that 2,000 V/cm is the electric field strength required to permit the transmembrane potential to surpass the electroporation threshold of the innermost lipid layer (Tarek 2005). Despite the ability to generate free chlorine in PBS during electroporation, the concentration was negligible, 0.4 mg/L which did not significantly contribute to the inactivation results. Furthermore, chlorination of the samples occurred more than 1 min post-electroporation which indicates that despite the amount of time needed for pores to reseal, inactivation occurred because the *A. suum* eggs were successfully, and potentially irreversibly, porated.

Two aspects of this research paper are important to note due to their impact on the experimental method. First, the effects of electrolysis during electroporation must be considered to impartially evaluate the results presented here. Although electrolysis occurs, as observed by bubbles forming on the electrode surface, the overall impact of the



products formed on *A. suum* inactivation is considered to be negligible and has a second-order effect (Sale & Hamilton 1967; Neu & Neu 2009). This is supported by the pore creation concept, which indicates that the current passing through the pores varies based on the size of their radii, which is the result of the transmembrane potential responding to the pulsed electric field. Therefore, the effects of the applied electric field are of more importance in determining the exact causes of inactivation. Next, the amount of energy required for the transmembrane potential to surpass the electroporation threshold differs based on the composition of the lipid bilayer. Existing bonds between lipids and membrane-bound proteins must be broken to decrease membrane stability before pores can form (Tarek 2005). Therefore, *A. suum* eggs may require a higher energy input to prompt the poration event because the lipid layer is composed of ascarosides (Mei et al. 1997; Dalton et al. 2006; Choe et al. 2012; Naidoo et al. 2017).

## CONCLUSIONS

This research explores the first known use of electroporation to effectively permeabilize the eggshell and terminate the embryonic development of *A. suum*. These experimental findings demonstrate for the first time that electroporation, using a pulsed electric field of 2,000 V/cm, coupled with a short period of exposure to a low concentration of free chlorine (10 mg/L) or hydrogen peroxide (3%), can successfully deactivate *A. suum*. Further experimentation examining the use of higher applied electric fields, with varying pulse durations, coupled with different concentrations of free chlorine is underway. By adding different concentrations of reactive free chlorine, the researchers hope to identify inactivated *A. suum* eggs amongst all electroporation parameters. These experiments will inform the identification of optimal treatment parameters, as a dose-response relationship, with the goal of achieving *A. suum* inactivation with lower voltage amplitudes, thus reducing total energy required for inactivation, in conjunction with varying concentrations of oxidizing agents. These parameters will inform the design and evaluation at scale of a treatment system that may ultimately be deployed as a back-end treatment option for the destruction of *A. suum*.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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