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RESEARCH ARTICLE

Ozone and peroxone disinfection of *Toxocara canis* eggs in water

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ABSTRACT

Water pollution in developing countries continues to be a major health problem due to various anthropological activities that contribute to the spread of many parasitic diseases, including those caused by helminths. The aim of this study is to explore the ability of ozone and peroxone to disinfect drinking water contaminated samples with *Toxocara canis* eggs. The oxidants used were ozone and ozone-hydrogen peroxide combination. The treatment of *Toxocara canis* eggs was carried out in a 50 ml reactor with an operating volume of 10 ml. The pH conditions (5, 7 and 10) were varied for each treatment. The treatment effect was calculated by counting eggs and examining the condition of the larvae larval condition (whole, broken and hatched larvae) using an optical microscope. The experiment was carried out by exposing the eggs for 60 and 120 minutes to ozone and peroxone. The best results were obtained for helminths treated with the ozone/hydrogen peroxide combination at pH 10, with an inactivation of 79.2%. The synergistic effect of ozone combined with hydrogen peroxide allows higher helminth egg inactivation rates, demonstrating that advanced oxidation processes are a real alternative to apply in the inactivation of *Toxocara canis* eggs. The results obtained in this study show that the ozone and peroxone treatment could be a useful disinfection process to destroy or inactivate *Toxocara canis* eggs in processes commonly applied in water treatment.

Keywords: Drinking water; ozone; water treatment; emerging contaminants; *Toxocara canis*.

INTRODUCTION

Water pollution in developing countries due to various anthropogenic activities such as indiscriminate disposal of municipal and industrial waste, large-scale applications of chemicals in agriculture as well as the prevailing ecological conditions and nutritional deficiencies in these countries favour the existence of many parasitic diseases, including those caused by helminths (Lamothe & Garcia, 1998). It is widely distributed worldwide and is estimated to infect tens of millions of people each year with an estimated seroprevalence of 19% (Fakhri *et al*., 2018). *Toxocara canis* (Nematode: *Ascaridida*) is the causative agent of toxocariasis (Despommier, 2003), which is considered among the neglected parasitic infections with public health priority (Chen *et al*., 2018; Timothy & Dwight, 2022). In addition to dogs, *T. canis* can also infect a wide variety of paratenic hosts, including humans (Baaten *et al*., 2011; Lee *et al*., 2016). Disease in humans can manifest in two forms, visceral larva migrans (VLM) and ocular larva migrans (OLM) (Lamothe & Garcia, 1998). Infection in humans occurs when food or water contaminated with embryonated eggs is consumed, hatching in the gut, and releasing second instar larvae (Soulsby, 1987; Quiróz, 1997). The other mode of infection is by ingestion of larvae in the viscera and uncooked meat from infected paratenic hosts (Taira *et al*., 2004). *Toxocara canis* eggs are extremely resistant to the climatic environment, which facilitates infection of both animals and humans (Gamboa, 2005; Morrondo *et al*., 2006). Potable and wastewater can be treated by conventional biological or physico-chemical processes. However, these processes are not sufficient to eliminate or inactivate pathogenic helminths or protozoa, so special disinfection treatments are necessary to eliminate these pathogens.

Advanced oxidation processes involve the generation and use of transition chemical species with high oxidising power such as the OH radical, which has a high oxidising effectiveness on organic matter and is considered an effective disinfectant (Orta de Velásquez *et al*., 2017). The use of Advanced Oxidation Processes with ozone and hydrogen peroxide is a good option to eliminate helminth eggs, especially those that can resist the most used disinfectants, such as chlorine (Hernández, 2003). In this research we test the application of Advanced Oxidation Processes on water samples, using ozone as oxidant and the ozone-hydrogen peroxide combination at different pH conditions, for the destruction or inactivation of *Toxocara canis* eggs, and we evaluated the morphological alterations of the eggs by optical microscopy and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Collection of *T. canis* eggs

Adult female forms were obtained by direct extraction from necropsies of dogs less than 6 months old. Five to six adult forms were crushed with a tissue homogeniser and *T. canis* eggs were obtained. The eggs were separated from the organic matter by centrifugation at 500 rpm for 7 min and washed 3 times with 0.8% saline solution. From this suspension, eggs were then counted to determine eggs/ml, excluding from the count number those eggs which, when observed under the microscope, did not reach maturity, or contained deformed embryos in the control sample. Aliquots of 1 ml with 2200±20 eggs/ml were obtained for subsequent treatments at different pH (5, 7 and 10) with ozone and peroxone (ozone/hydrogen peroxide).

Reactor design and operating conditions

A mini glass reactor was designed for the treatment of *Toxocara canis* eggs in a vertically elongated shape with a volumetric capacity of 50 ml and an operating volume of 10 ml (Figure 1). Ozone treatment was continuous by using a reactor diffusion system to keep the ozone dissolved in the water throughout the process. The operating conditions were at different pH of 5, 7 and 10, ambient temperature and pressure, and the reaction time was 60 and 120 minutes. Ozone was generated at 0.7 mg O_3/min (for 60 min) and 1.1 mg O_3/min (for 120 min) with an ozone generator (Ozein 19L New Electric, MEX). The amount of dissolved ozone in contact with the eggs was measured by the indigo colorimetric method, the TC factor was 42 mg/L*min and 132 mg/L*min, respectively (Eaton *et al*., 2005). The O_3/H_2O_2 molar ratio was 0.5M/0.0035 M, with a TC of 15.1 mg/L* min. Standard Methods (APHA, AWWA, WEF, 1992) were applied for water analysis.

Treatments

Ozone

For each test, 1 ml of *T. canis* egg stock (2200±20) and 9 ml of solutions at different pH were placed in the ozone reactor. Control tests were performed under similar conditions, but the samples were in contact with air, briefly, in the mini glass reactor, the *Toxocara canis* eggs were placed 10 ml volume, then put in contact with air.

Peroxone

Eggs were treated under the same conditions as the ozone treatment, but instead of ozone, 0.33 ml of 30% hydrogen peroxide and 8.67 ml of solutions at different pH were placed in the reactor. For the control tests, the eggs were only in contact with air. After the corresponding treatments the egg suspensions were centrifuged at 500 rpm for 7 min and washed 3 times with 0.8% saline solution pH 7. The eggs were then suspended in 1 ml of water pH 7 and counted under the light microscope. The treated egg samples were processed for analysis by light microscopy, scanning electron microscopy (SEM).

Evaluation of *T. canis* eggs by light microscopy

A control of *T. canis* eggs without treatment and after oxidation with ozone and O_3 -H_2O_2, was prepared for observation by light microscopy and scanning electron microscopy to compare the effect of the oxidants on the helminth eggs.

Scanning Electron Microscopy (SEM)

Eggs from each treatment were fixed with 3% glutaraldehyde (EM grade) at 4°C for 2 h and then washed 3 times, 30 min each, with washing solution containing 0.15 M phosphate buffer, pH 7.4, 5% sucrose and 0.5 mM calcium chloride (Ooi *et al*., 1998). Eggs were then fixed with 1% osmium tetroxide (EM grade) for 2 h. Eggs were dehydrated in a dryer for 2 hours. The eggs were dehydrated in a series of 10% to 100% ethanol solutions, 15 min each step and dried at the critical point using CO_2 for 7 min and then the samples were ionised in the Denton Vacuum DESK II ioniser. Eggs were observed under a JEOL JsM-5800LV scanning electron microscope and images were taken at 1900 -2500X magnification.

Inactivation and viability of *Toxocara canis* larvae

The inactivation effect of ozone and ozone/hydrogen peroxide combination was performed by using 1% Methylene Blue dye in aqueous solution (Shira *et al*., 2011). Determinations were made

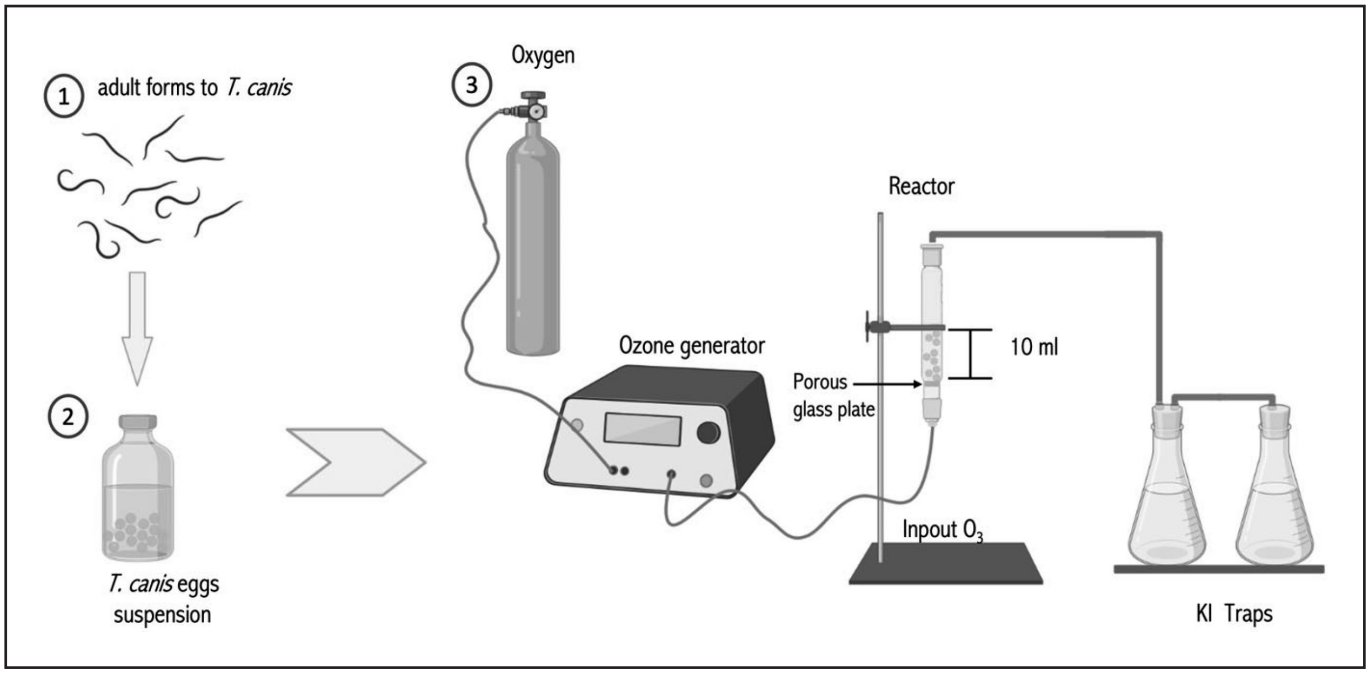

Figure 1. *T. canis* egg treatment diagram. 1: Five to six adult forms to *T. canis* were crushed with a tissue homogeniser and the eggs were then obtained. 2: The eggs were separated from the organic matter by centrifugation. In this suspension, the eggs were counted to determine eggs/ml. Aliquots of 1 ml with 2200±20 eggs/ml were obtained for subsequent treatments conditions. 3: A mini glass reactor was designed for the treatment of *Toxocara canis* eggs in a vertically elongated shape with an operating volume of 10 ml. The ozone treatment was continuous by using a reactor diffusion system to keep the ozone dissolved in the water throughout the process.

by direct observation under an inverted microscope. The effects of oxidant treatments on the morphology of *T. canis* eggs, as well as embryological hatching and survival of second instar larvae of *T. canis* larvae were observed. Viability was expressed as a percentage.

Statistical analysis

Two-way ANOVA tests were used to compare the inactivation (%) of eggs to 60 and 120 minutes. Analyses included fixed effects for pH (5, 7 and 10), treatments (ozone and peroxone), and treatments'pH interactions. If the interaction was statistically significant, post hoc analysis using the Sidak correction method for mean differences was performed to determine whether the changes in inactivation between the pH were statistically significant between ozone and peroxone treatments. The Shapiro–Wilk test was applied to verify the normality of the residual. SPSS software version 21 (IBM, Armonk, NY, USA) was used at the significance level 0.05.

RESULTS

At 60 and 120 minutes, the mean and standard deviation of inactivation (%) of eggs for pH of 5, 7, and 10 to ozone and peroxone are reported in Table 1. To analyze whether the inactivation (%) of eggs depends on the treatment and the pH, two-way ANOVA tests were performed. Hence, at 60 minutes, the analysis shows the following for the fixed effects of pH (F=756.93, df=2, η^2 =0.992, and P<0.001) and treatment (F=29.09, df=1, η^2=0.708, and P<0.001), and treatments×pH interaction (F=23.78, df=2, η^2=0.799, and P<0.001). At 60 minutes, post hoc analysis of treatments×pH interaction showed that the inactivation with pH of 5, 7, and 10 was statistically different between treatments, respectively. In other words, the results show that at higher pH then the inactivation of eggs is greater, and the inactivation is greater when peroxone is used compared to ozone, as shown in Figure 2a). Similarly, for ANOVA analysis to 120 minutes, we have the fixed effects of pH (F= 80.13, df=2, η^2 =0.930, and P<0.001) and treatment (F=2477.77, df=1, η^2=0.995, and P<0.001), and treatment at different pH interaction (F=8.35, df=2, η^2=0.582, and P=0.005). Figure 2b) shows that the inactivation is much higher when peroxone is used compared to ozone for any pH, and the inactivation of peroxone is much higher at alkaline pH than at acidic pH The results of ozone oxidation of *T. canis* eggs show that inactivation 40.9% (SD 1.38) was better at 120 minute treatment and pH 10, compared to that obtained at 60 min under the same conditions (difference of means 5.5, t = 4.95, df = 4, P=0.007, 95% IC=2.41 to 8.58). The lowest inactivation 16.5% (SD 0.87) was obtained with a 60 minute treatment at acidic pH. The highest inactivation value for *T. canis* eggs was 79.2% (SD 1.53) using the O_3/H_2O_2 combination at pH 10 and 120 minutes of oxidation. The combination of ozone with hydrogen peroxide reported better inactivation results of helminth eggs at alkaline pH, due to better

Table 1. Results of the inactivation of *T. canis* eggs oxidized with ozone and ozone-hydrogen peroxide

	Oxidation with ozone		**Oxidation with ozone/peroxide**	
	CT mg/L* min		Molar relation O3/H2O2 0.5/0.0035	
	42	132		
	Oxidation time (min)			
pH	60	120	60	120
	Inactivation (%)			
5	16.5 (0.87)	34.1 (1.32)	14.6 (0.72)	67.8 (1.93)
7	20.3 (0.96)	34.8 (0.72)	25.9 (0.62)	66.3 (1.63)
10	35.4 (1.34)	40.9 (1.38)	39.2 (1.18)	79.2 (1.53)

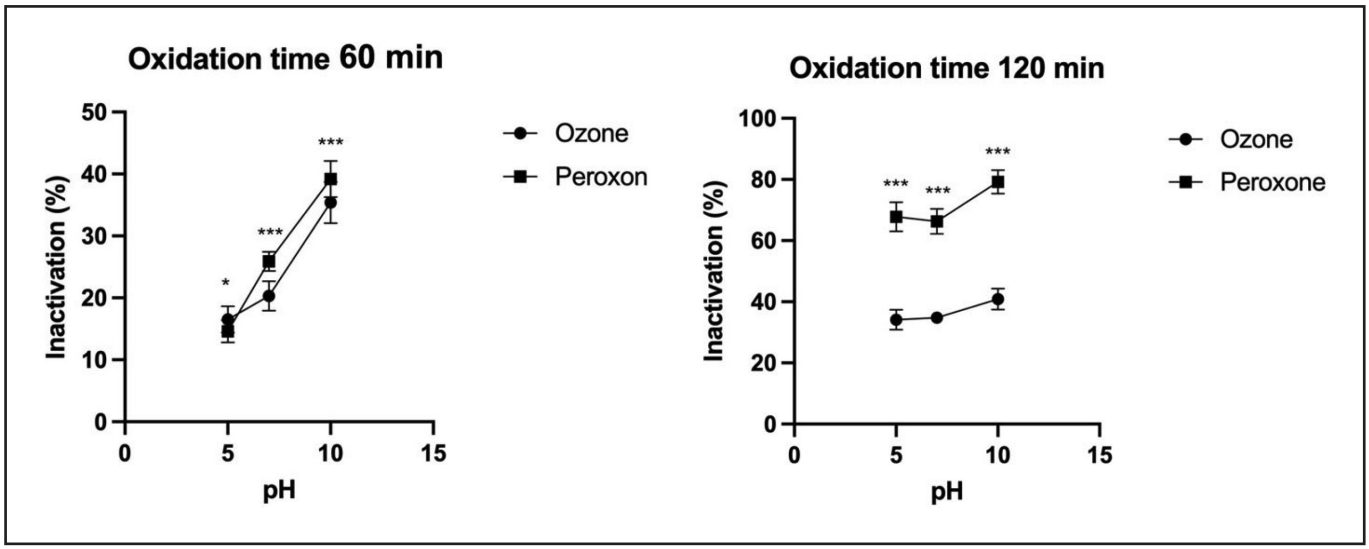

Figure 2. Inactivation of eggs at 60 and 120 min, showing that the ozone and peroxone increases the inactivation with an alkaline pH, but the inactivation was much higher in alkaline pH and in 120 minutes. *P<0.05, **P<0.01 and ***P<0.001. Values expressed as the means ± 2SEM were evaluated by two-way ANOVA.

production of OH- radicals (Gottschalk *et al*., 2000). The effects of oxidation of *T. canis* eggs with ozonisation and hydrogen peroxide-ozone by light and electron microscopy, and the morphological changes and ruptures of *T. canis* egg layers can be observed (Figures 3 and 4). Figure 3 shows optical microscopy photographs, where *T. canis* eggs can be observed in their normal state and the effect produced in the egg layers after each oxidant treatment, where the rupture of the layer and expulsion of the cellular content can be observed, so that the inactivation or destruction of the *T. canis* eggs is produced. Figure 4 shows micrographs of *T. canis* eggs by scanning electron microscopy, shown in their original form and after ozone and ozone-hydrogen peroxide treatments, where the effects of the oxidants on the egg layers can be observed, which can lead to inactivation or destruction of the eggs. These micrographs show the difference in the effects of the oxidants. According to SEM images, the ozone/hydrogen peroxide combination combines the egg disintegration mechanisms of the two oxidants.

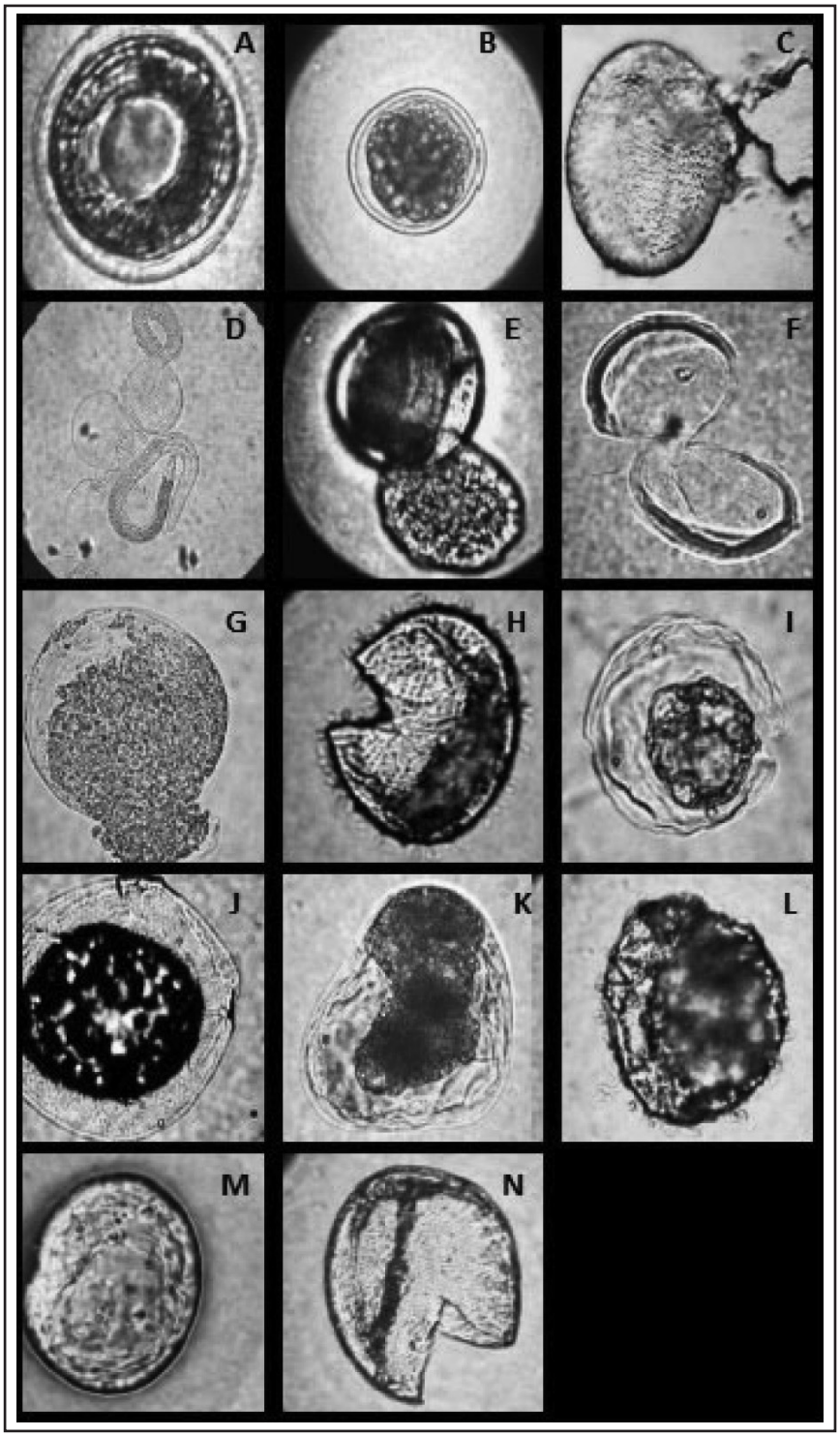

Figure 3. Optical microscopy photographs, where the *T. canis* eggs can be observed in their normal state and the effect produced in the eggs' layers after each oxidant treatment, where the rupture of the layer and expulsion of the cell content, can be observed, thus producing the inactivation or destruction of *T. canis* eggs. A-F: *T. canis* eggs without treatment; G-I: *T. canis* eggs with O_3 treatment; J-L: *T. canis* eggs with O_3/H_2O_2 treatment. M-N: *T. canis* eggs with H_2O_2 treatment.

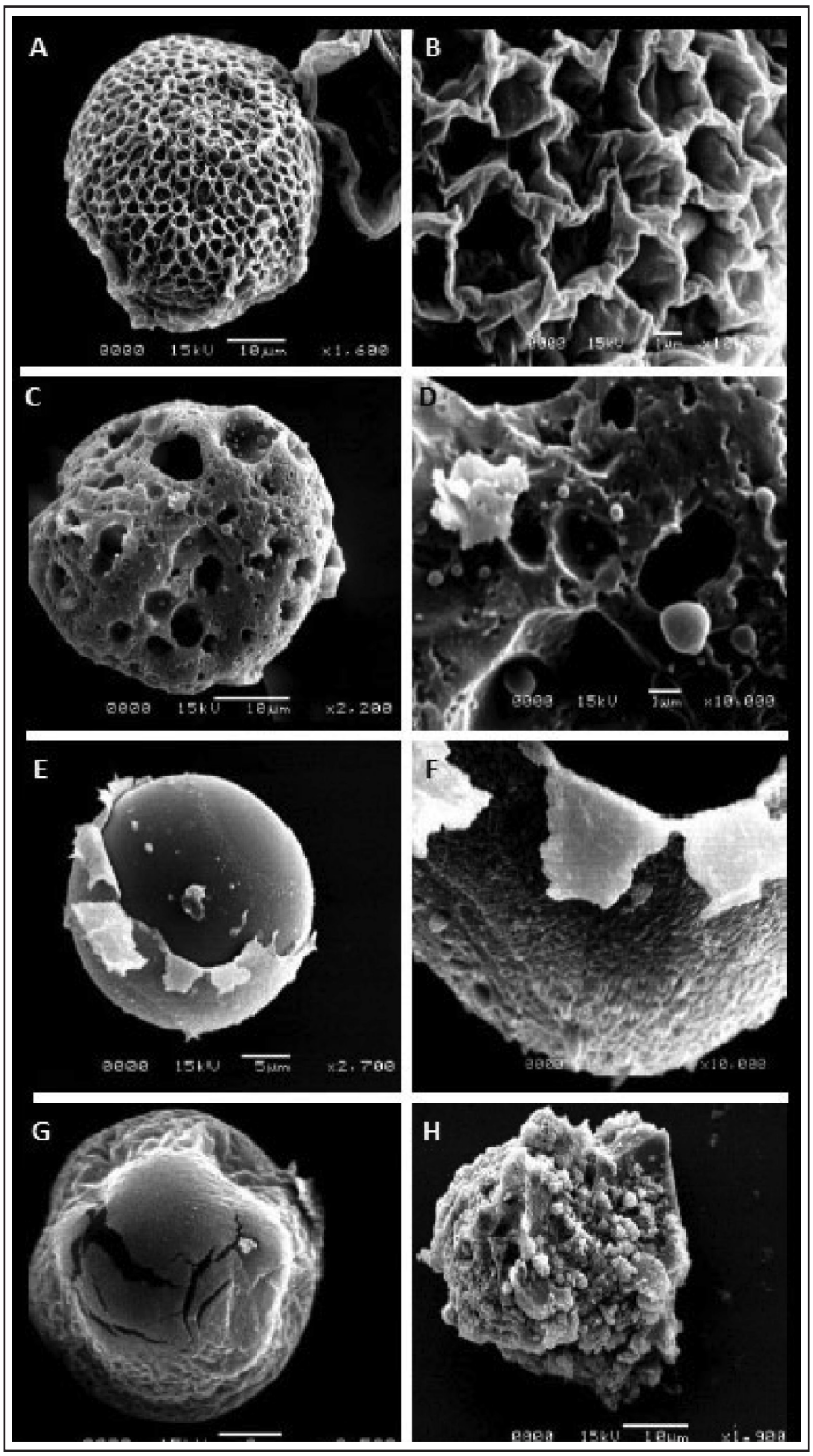

Figure 4. Electronic microscopy photographs of *T. canis* eggs are shown in their original shape and after ozone and ozone-hydrogen peroxide treatment, where the effects of the oxidants in the eggs' layers can be observed, which produces their inactivation or destruction, measured as % of inactivation of the *T. canis* eggs. In these photographs the difference of the effects of the oxidants can be observed, on the one hand the direct reaction of the ozone, selective reaction, with an even destruction of the egg's layers and on the other hand the reaction of oxidation by radicals, with the peroxide, non-selective reaction, with a destruction in an irregular way in the diverse components of the egg. A-B: T*. canis* eggs without treatment; C-D: *T. canis* eggs with H_2O_2 treatment; E-F: *T. canis* eggs with O_3 treatment; G-H: *T. canis* eggs with O_3/H_2O_2 treatment.

DISCUSSION

Water contamination with pathogens continues to cause serious diseases worldwide. Due to the potential risk of chlorine-resistant microorganisms, research on alternative disinfection processes using ozone, UV, chlorine dioxide or combined processes has become a matter of public concern (Koivunen & Heinonen-Tanski, 2005; Li *et al*., 2013; Ofori *et al*., 2018). Different studies have shown that ozonation is often effective, fast and is considered a promising disinfection method for the treatment of drinking water, especially for the inactivation of pathogens such as bacteria, viruses, and fungi.

Advanced oxidation processes involve the generation and use of transition chemical species with high oxidising power such as the OH-radical, which has a high oxidising efficiency on organic matter and is considered an effective disinfectant. When ozone decomposes in water, the hydrogen peroxide and hydroxyl free radicals formed have a high oxidising capacity and play an active role in the disinfection process (EPA, 1999). The effectiveness of disinfection depends on the sensitivity of the organisms to be treated, the contact time and the ozone concentration.

In this study, we showed that the effectiveness of ozone is favoured under alkaline pH conditions, reacting indirectly to helminth eggs through reactions involving the formation of free radicals, mainly OH-radicals.

Electron microscopy images show that ozone acts regularly on the gradually degraded egg layer. In the case of the ozone/hydrogen peroxide combination, it proved to be the most effective treatment for inactivation of *Toxocara canis* eggs. This mixture has a greater oxidative effect on helminth eggs. According to the electron microscope images, the ozone/hydrogen peroxide combination combines the egg disintegration mechanisms of the two oxidants, i.e., the breakdown occurs in layers, and the formation of cracks reaching the inside of the egg, the destruction being more effective and in less time. In conclusion, *Toxocara canis* eggs, which have a high resistance mainly to environmental stresses, were successfully inactivated by ozone and ozone/peroxide treatment with acceptable treatment times or TC values. The photographs clearly show the difference between the direct action of ozone, as molecular ozone, under acidic pH conditions, indicating a selective reaction on the egg components, with a regular destruction of the surface layer, while the non-selective action of the peroxide shows the destruction of the egg in an irregular way, by the action of OH- and O_2H-radicals among others. Some authors have reported the effect of ozone disinfection on several pathogenic microorganisms, being able to destroy pathogenic organisms present in water samples, such as *Acanthamoeba* spp, *Naegleria* spp, *A. castellanii*, and *E. coli* (Cursons *et al*., 1980; Thomas *et al*., 2008; Lee *et al*., 2016), to decrease the count of some viruses, such as Norwalk Virus, Poliovirus 1 and Bacteriophage MS2 (Shin & Sobsey, 2003), and in other protozoa that contaminate water like *Giardia, Cryptosporidia, Microsporidia, Cyclospora and Blastocystis* (Khalifa *et al*., 2001). Ibסבez-Cervantes et al., demonstrated that with peroxone at pH 10 the *Hymenolepis nana* eggs lost their outer coat, making them more vulnerable (Ib סבez-Cervantes *et al*., 2013). The use of ozone as a disinfectant agent for media other than water has also been demonstrated as an alternative process for the decontamination of N95 masks and biosafety gowns against COVID-19, ESKAPE bacteria, and other devices (Ibáñez-Cervantes *et al*., 2022).

CONCLUSION

The efficacy of ozone is favoured at alkaline pH, reacting indirectly with helminth eggs through reactions involving the formation of free radicals, mainly -OH radicals. The synergistic effect of ozone combined with hydrogen peroxide, allows obtaining higher percentages of inactivation of helminth eggs, demonstrating that POAs are a real alternative to apply in the inactivation of helminth eggs of *Toxocara canis*. The main variables that directly affect these oxidation processes are pH, temperature, degree of mixing (type of reactor or contactor), ozone concentration and contact time. The application of these oxidation technologies involves determining the operating conditions for each given case, i.e., it depends on the empirical results to be applied in each treatment and thus have the most accurate and reliable data to be used. This oxidation affected the surface of the eggs, caused by the chemical reaction of the oxidants with the organic compounds of the surface eggs. The action of the two oxidants, ozone peroxide, shows an increase in radical activity, with greater destruction of the eggs. However, it is important to mention as a limitation of our study; that tests are required on the reduction of the infective capacity of unhatched eggs treated with ozone and peroxone and that are observed apparently well, they can infect their host. This limitation can be addressed in future research, carrying out infectivity tests of *T. canis* eggs treated with ozone and peroxone in animals' models.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

REFERENCES

Abou-El-Naga, I.F. (2018). Developmental stages and viability of *Toxocara canis* eggs outside the host *Biomedica* **38**: 189-197. https://doi.org/10.7705/biomedica.v38i0.3684

APHA, AWWA, WPCF. (1992). Métodos normalizados para el análisis de aguas potables y residuales (In Spanish). **4**: 183-187.

Baaten, G.G., Sonder, G.J., van Gool, T., Kint, J. A. & van den Hoek, A. (2011). Travel-related schistosomiasis, strongyloidiasis, filariasis, and toxocariasis: the risk of infection and the diagnostic relevance of blood eosinophilia. *BMC Infectious Diseases* **11**: 84. https://doi.org/10.1186/1471-2334-11-84

Despommier, D. (2003). Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Reviews* **16**: 265-272. https://doi.org/10.1128/CMR.16.2.265-272.2003

Chen, J., Liu, Q., Liu, G.H., Zheng, W.B., Hong, S.J., Sugiyama, H., Zhu, X.Q. & Elsheikha, H.M. (2018). Toxocariasis: a silent threat with a progressive public health impact. *Infectious Diseases of Poverty* **7**: 59. https://doi.org/10.1186/s40249-018-0437-0

Cursons, R.T., Brown, T.J. & Keys, E.A. (1980). Effect of disinfectants on pathogenic free-living amoebae: in axenic conditions. *Applied and Environmental Microbiology* **40**: 62-66. https://doi.org/10.1128/aem.40.1.62-66.1980

Eaton, A.D., Clesceri, L.S., Rice, E.W. & Greenberg, A.E. (2005). Indigo colorimetric method for residual ozone analysis. *Standard Methods for the Examination of Water and Wastewater: Method* **4500-O3**: 44-146.

Environmental Protecction Agency (EPA). (1999). Manual de orientación EPA, oxidantes y desinfectantes alternativos. Chapter VII.

Fakhri, Y., Gasser, R.B., Rostami, A., Fan, C.K., Ghasemi, S.M., Javanian, M., Bayani, M., Armoon, B. & Moradi, B. (2018). *Toxocara eggs* in public places worldwide – a systematic review and meta-analysis. *Environmental Pollution* **242**: 1467-1475. https://doi.org /10.1016/j.envpol.2018.07.087

Gamboa, M.I. (2005). Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. *Journal of Helminthology* **79**: 327-331. https://doi.org /10.1079/joh2005287

Gottschalk, C., Libra, J.A. & Saupe, A. (2000). Ozonation of water and waste water. Germany, Weinheim: Wiley Vch.

Hernández C. (2003). "Aplicación de ozono para inactivar bacterias coliformes y huevos de helminto en agua residual". UAM – Azcapotzalco.

Ibáñez-Cervantes, G., Ramírez-Cortina, C.R., Marquez-Navarro, A., Alonso-Gutiɪrrez, M.S., León-Ãila, G., León-García, G. & Nogueda-Torres, B. (2013). Effect of ozone and peroxone on helminth *Hymenolepis nana* eggs. *Ozone: Science and Engineering* **35**: 201-207. https://doi.org/10.1080/01919512.2013.771762

Ibáñez-Cervantes, G., Lugo-Zamudio, G.E., Cruz-Cruz, C., Durán-Manuel, E.M., Bravata-Alcántara, J.C., García-Moncada, E., Mata-Rocha, M., Delgado-Balbuena, L., Cureno-Díaz, M.A., Ramírez-Cortina, C.R. *et al*. (2022). Ozone as an alternative decontamination process for N95 facemask and biosafety gowns. *Materials Letters* **311**: 131554. https://doi.org/10.1016/j.matlet.2021.131554

Ibáñez-Cervantes, G., Cruz-Cruz, C., Durán-Manuel, E.M., Loyola-Cruz, M.A., Cureno-Díaz, M.A., Castro-Escarpulli, G., Lugo-Zamudio, G.E., Rojo-Gutierrez, M.I., Razo-Blanco Hernandez, D.M., Lopez-Ornelas, A. *et al*. (2023). Disinfection efficacy of ozone on ESKAPE bacteria biofilms: potential use in difficult-to-access medical devices. *American Journal of Infection Control* **51**: 11-17. https://doi.org/10.1016/j.ajic.2022.03.037

Khalifa, A.M., El Temsahy, M.M., Abou, E.I. & Naga, I.F. (2001). Effect of ozone on the viability of some protozoa in drinking water. *Journal of the Egyptian Society of Parasitology* **31**: 603-616.

Koivunen J, Heinonen-Tanski H. (2005). Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. *Water Res.* **39**: 4445-53.

Lamothe, A.R. & Garcia, P.L. (1998). Helmintiasis del hombre en México. México (D.F.): AGT, pp. 1-128.

Lee, Y., Imminger, S., Czekalski, N., von Gunten, U. & Hammes, F. (2016). Inactivation efficiency of *Escherichia coli* and autochthonous bacteria during ozonation of municipal wastewater effluents quantified with flow cytometry and adenosine tri-phosphate analyses. *Water Research* **101**: 617-627. https://doi.org/10.1016/j.watres.2016.05.089

Li, D., S. Zeng, A. Z. Gu, M. He and H. Shi. (2013). Inactivation, reactivation and regrowth of indigenous bacteria in reclaimed water after chlorine disinfection of a municipal wastewater treatment plant. *Journal of Environmental Sciences*. **25**: 7. 1319-1325.

Morrondo, P., Díez-Morrondo, C., Pedreira, J., Díez-Baños, N., Sánchez-Andrade, R., Paz-Silva, A. & Díez-Baños, P. (2006). *Toxocara canis* larvae viability after disinfectant-exposition. *Parasitology Research* **99**: 558-561. https://doi.org/10.1007/s00436-006-0200-5

Ofori, I., Maddila, S., Lin, J. & Jonnalagadda, S.B. (2018). Chlorine dioxide inactivation of Pseudomonas aeruginosa and Staphylococcus aureus in water: The kinetics and mechanism. *Journal of Water Process Engineering*. **26**: 46-54.

Ooi, H.K., Lin, C.L. & Wang, J.S. (1998). Effect of ozone treatment on *Toxocara canis* eggs. *Journal of Veterinary Medical Science* **60**: 169-173. https://doi.org/10.1292/jvms.60.169

Orta de Velásquez, M.T., Yáñez-Noguez, I., Casasola-Rodríguez, B. & Roman-Roman, P.I. (2017). Effects of ozone and chlorine disinfection on VBNC *Helicobacter pylori* by molecular techniques and FESEM images. *Environmental Technology* **38**: 744-753. https://doi.org/10.1080/09593330.2016.1210680

Quiróz, R.H. (1997). Parasitología y enfermedades parasitarias de los animales domésticos. México: Limusa, pp. 404-412.

Shin, G.A & Sobsey, M.D. (2003). Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. *Applied and Environmental Microbiology* **69**: 3975-3978. https://doi.org/10.1128/AEM.69.7.3975-3978.2003

Shira, C.S., Sorvillo, F.J., Sorvillo, T. & Eberhard, M.L. (2011). Viability of *Baylisascaris procyonis* eggs. *Emerging Infectious Diseases* **17**: 1293-1295. https://doi.org/10.3201/eid1707.101774

Soulsby, E.J.L. (1987). Parasitología y enfermedades parasitarias de los animales domésticos. 7× edición. México: Interamericana, pp. 150-155.

Taira, K., Saeed, I., Permin, A. & Kapel, C.M.O. (2004). Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. *Veterinary Parasitology* **121**: 115-124. https://doi.org/10.1016/j.vetpar.2004.01.018

Timothy, K.W. & Dwight, D.B. (2022). *Toxocara canis*. *Trends in Parasitology* **38**: 709-710. https://doi.org/10.1016/j.pt.2022.01.002

Thomas, V., Loret, J.F., Jousset, M. & Gilbert, G. (2008) Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. *Environmental Microbiology* **10**: 2728-2745. https://doi.org/10.1111/j.1462-2920.2008.01693.x