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Effect of mineral aggregates on the morphology and viability of *Toxocara canis* eggs

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ABSTRACT

This work shows the effects on morphology and inactivation or viability loss on *Toxocara canis* eggs (TCE) as a result of their direct exposure to mineral aggregates (MA). Contact assays were performed on TCE-MA in flasks using 15 TCE per sample, and MA (0.01, 0.05, 0.1, 0.5, 1, and 2 g mL^{-1}) for exposure times of 30, 60, 90, and 120 min, under shaking (US), and no shaking (NS) conditions. The MA characterization was performed by X-Ray Diffraction Spectroscopy (XRDS), Mass Absorption Spectroscopy with Graphite Furnace (MASGF), Coupled Plasma Mass Spectrometry (ICP-MS), and X-Ray Fluorescence Spectrometry (XRF). MA are composed of hematite, sandinite, montmorillonite, calcite, quartz, nimite, and jordanite. The composition of the residue on ignition (mg kg^{-1}) was: CaO (91,746.0 ± 1913.7), Zr (2542.8 ± 131.2), Al_2O_3 (1683.0 ± 209.3), SiO_2 (1138.0 ± 123.5), as well as Fe_2O_3 (212.0 ± 23.1), Cu (0.2 ± 0.4), TiO_2 (36.4 ± 6.1), and Ag (128.0 ± 1.4). In order to identify the morphological alterations associated with the TCE viability loss in the contact assays, photo-micrographs were taken by an optical microscope (OM) and a scanning electron microscope (SEM). These experiments showed structural damage on the external and internal layers (nucleus and hyaline solution) of the TCE, indicating a direct relationship with the viability loss or inactivation; the observed values were in the range of 35% (0.01 g of 2 mm MA; 30 min; NS) up to 92% (2.00 g of 0.6 mm MA; 120 min; US). The viability loss increases as the MA particle size decreases, and as the MA concentration increases, the effect of the time of contact and the shaking condition also affect the viability loss. The external morphological damage observed on TCE includes impairment, thinning, and fracture of the external layers. On the internal structures, heterogeneity of the hyaline solution and release of the nuclear material were observed. Consequently, the TCE viability loss can be attributed to the structural damage caused by the mechanical stress produced by direct contact with the MA surface. This damage is synergistic with the interaction caused by the chemical stress between the TCE biomass and the MA. Certain compounds of the MA, containing Ag, Cu, Fe, Al, Ti and Zn, have been reported as having biocidal ability.

1. Introduction

Morbidity associated with helminthiasis is a global public health problem, emphasized in developing countries due to poverty, causing an estimate of three and a half million deaths per year (Papale et al., 2008). The precarious conditions on urbanistic infrastructure and the use of untreated wastewater to irrigate crops that are later eaten uncooked are contributing factors to the prevalence of these conditions (Maya et al., 2012; Jimenez-Cisneros and Maya-Rendon, 2007; De Victorica and Galván, 2003).

Municipal wastewater in developing countries has been reported with a range of 70–3000 HE L^{-1}. In Mexico, 6–98 HE L^{-1} in metropolitan areas, up to 330 HE L^{-1} in rural and peri-urban areas (Jimenez-Cisneros and Maya-Rendon, 2007; Jimenez and Wang, 2006), while France and USA reported less than 10 HE L^{-1}

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(Jimenez-Cisneros, 2008). WHO recommends a criterion of ≤1 HE L^{-1} for irrigation wastewater for crops intended for fresh consumption. However, epidemiological studies in Central Mexico indicate that the restriction of ≤1 HE L^{-1} may not be sufficiently protective in situations where conditions favor egg survival (WHO, 2006).

Commonly used methods for wastewater disinfection as chlorine and ultraviolet radiation have not been effective to eliminate certain microorganisms nor highly resistant structures of propagation, such as helminth eggs (HE) (Mun et al., 2009). This is relevant issue to health due to the long survival times (several months) that largely exceed the time periods reported for other organisms, and also due to their higher resistance to inactivation. Aladawi et al. (2006) state that applying ultraviolet (UV) radiation at 0.0085–15.37 J cm^{-2} doses to *Ascaris lumbricoides* eggs shows no lethal effect, on the contrary, it stimulates the embryonic period (transformation into the infectious form), and accelerates the larval stages (intermediary development stages between egg and adult). On the other hand, Guadagnini et al. (2013) claim that the UV radiation has an inactivation capacity of 82% on HE. However, the combination of UV radiation (0.0070 J cm^{-2}) and H_2O_2 (30 mg L^{-1}) leads to 81% inactivation. Therefore, there is a need of further research on the effects that other agents produce on non-larval eggs, and the embryonic process. On the other hand, ozone has shown to produce up to 95% impairment of HE viability when used for a time period of 45–180 min (Gadomska et al., 1991) and 94% impairment when used in 4.6 mg O_3 L^{-1} for 60 min (Rojas-Valencia et al., 2004). However, ozone being non-selective is needed at high dosage in raw wastewater and/or a pre-treatment is needed to remove organic matter (Orta de Velásquez et al., 2004). In this sense, Sobsey (2002) states that HE have structures that are more resistant compared to the structures commonly present in certain bacteria, viruses, and protozoa.

Particularly, *Toxocara canis* eggs (TCE) are around 85 μm, have a subglobose form with an irregular envelope, and the protoplasm has a granular appearance (Rodríguez et al., 2006). TCE have five layers, one or two more than the eggs of *Hymenolepus nana*, *Taenia saginata*, *Fasciola hepatica*, *Schistosoma* spp., *A. lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura* (Jimenez-Cisneros, 2008; Jimenez-Cisneros and Maya-Rendon, 2007; Habluetzel et al., 2003; Ayçiçek et al., 2001). The five-layered arrangement is as follows, from the most outer to the most inner layer: (1) a thin uterine membrane with small bulges; (2) a vitelline layer represented by a thin membrane that follows the contour of the crests and ridges of an underlying layer; (3) a thick homogeneous chitinous layer (up to 6.3 μm); (4) a granular electron-dense layer with an average thickness of 0.35 μm; and (5) a laminar zone formed by the superposition of four or five fibrous layers with an average thickness of 0.6 μm (Ayçiçek et al., 2001). This conformation confers the TCE a high structural resistance to unfavorable environmental and chemical conditions.

TCE were selected as a representative model of the experimental behavior of HE for the present work in view of their higher number of layers, their presence in municipal wastewater and for representing less human infection risk than other species. Also, the study of TCE acquires more relevance since it has been established that they are the agent that cause the conditions referred to as *ocular and visceral larva migrans* (Rodríguez et al., 2006). The presence of TCE has been reported on wastewater, feces, sewage in rural and urban areas, and represent a public health issue due to their prevalence and morbidity in humans (Maya et al., 2012; Dubná et al., 2007; Kozan et al., 2005; Habluetzel et al., 2003). With the aim of promoting the inactivation or viability loss of HE, other studies have addressed the use of solar and gamma radiation, as well as the addition of acid and cationic compounds. Also, different types of processes have been used to eliminate these reproductive structures, such as, aerobic, anaerobic and thermal processes, pH changes, fine filtration through membranes and zeolites, showing percentages of inactivation effectiveness ranging from 30% to 100% (Maya et al., 2012; Bandala et al., 2011; De Souza et al., 2011; Mun et al., 2009; Pecson et al., 2007; Vinnerås et al., 2003; Rivera-Garza et al., 2000). Similarly, assays have been performed on TCE using iodine, glutaraldehyde, benzalkonium chloride, potassium permanganate, ethyl alcohol, potassium hydroxide, phenolic solutions and a dihydroxybenzol and hydrogen peroxide solution resulting in partially favorable results (Shalaby et al., 2011; Ayçiçek et al., 2001). On the other hand, the use of metallic elements such as Ag, Fe, Cu, among others, either separately or combined, has also been applied showing a biocidal ability ranging from 50% to 90% on viruses, bacteria and fungi (Miranda-Ríos et al., 2011; Ruparelia et al., 2008; Dubas et al., 2006; Panacek et al., 2006; Morones et al., 2005; Sondi and Salopek-Sondi, 2004). However, there are no references regarding the effect of these elements on HE or TCE.

Mineral aggregates (MA) contain compounds with biocidal ability, such as Ag, Cu, Fe, Al, Ti and Zn. The use of MA may represent an alternative to induce viability reduction on HE. Additionally, the present work aims to explore the effect of the MA structure and metal contents upon their interaction with TCE. For this reason, in this work, special attention will be paid to the possible structural and physiological damage promoted by mechanical stress and the chemical interaction attributable to the presence of MA.

2. Materials and methods

The tested variables where the effect on morphology and on TCE viability loss derived from the contact with MA at different concentrations and exposure times. The *T. canis* eggs used during the assay were recovered by dissection of an adult female gravid uterus.

2.1. Physicochemical characterization of the MA

The MA used in this study were obtained from a mine located in Zacatecas, Mexico. These materials are considered inert rocks with no commercial value since they only contain traces of metallic elements. MA samples having a particle diameter (pd) of approximately 2.5 cm, were crushed with a Zenith-Shanghai JC250 × 1200 crushing equipment, and subsequently separated using sieves. There were two size fractions recovered; corresponding to 0.60 ± 0.05 mm and 2.00 ± 0.05 mm. Afterwards, the fractions were washed with running water to remove excess dust and dried on an oven at 250 °C in order to remove the remaining moisture. A sample of the recovered fractions was physicochemically characterized as follows: (a) Crystallography by X-Ray Diffraction Spectroscopy (XRDS) using a Siemens D5000 Diffractometer with a Cu tube, $k_\lambda = 1.0546$, and a Ni filter in an angular interval of 0–90° at 35 kV and 30 mA; (b) Mass Absorption Spectroscopy with Graphite Furnace (MASGF) according to ASTM E1613-94 (ASTM, 1997); (c) Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a ICP-Mass equipment, Bruker brand, Model Aurora M90 with coupled autosampler and computer, following the technical procedure PT-USAI-FQ-AA-001; and (d) X-Ray Fluorescence Spectrometry (XRF) using a SRS3000 spectrometer, where the determination of major elements was performed preparing a bead containing 67:33% of Lithium Tetraborate/Lithium Metaborate using the analytic program USAIM07. For minor elements, determination was made via a pressed sample with 10% wax, applying the analytical program Traza04 of the equipment itself.

2.2. Characteristics of the TCE

T. canis eggs (TCE) used in this work were provided by the Helminthology Department of the National School of Biological