Quick incubation process to determine inactivation of *Ascaris* and *Toxocara* eggs

C. Maya, M. Pérez, G. Velásquez, J. A. Barrios, A. Román and B. Jiménez

**ABSTRACT**

Helminth eggs are among the most important biological contaminants in environmental engineering. They pose a significant health risk associated with poor sanitation, the use of contaminated water for irrigation, and the disposal of excreta or sludge to land. Helminths are parasitic worms transmitted to humans via their eggs, which is the infective stage of their life cycle. They are therefore relevant to public health and environmental fields due to their low infectious dose, their persistence in the environment (up to several years), and their high resistance to conventional disinfection processes. The evaluation of the efficiency of any process of inactivation, through the determination of the viability of these parasites, is fundamental, but the traditional incubation technique requires 20 days to determine both the viability and the infectivity of nematode eggs. However, the present study found that, using an inactivation process at a temperature of 60°C for 1 hour and incubation at 28°C and 34°C, the absence of division of the nucleus of eggs of species from two genera, *Ascaris lumbricoides* and *Toxocara canis*, showed them to be inactivated following only 48 hours of incubation. Similar inactivation results were observed using an automatic system as long as the eggs were inactivated. The minimum time required to evaluate the inactivation of nematode eggs through the incubation technique was 48 hours.

**Key words** | *Ascaris* eggs, infectivity, larva, *Toxocara* eggs, viability

**INTRODUCTION**

In recent years, the reuse of wastewater and sludge for irrigation and agricultural application (even without treatment) has become an important practice due to its benefits (Jiménez 2006; Jiménez et al. 2007). The fertilization of land with wastewater could reduce or eliminate the need for commercial fertilizers (Jiménez et al. 2004, 2016). However, this practice also contributes to an increase in the incidence of waterborne diseases due to high concentrations of pathogens such as bacteria, protozoa, and helminths (WHO 2006). Helminths are relevant in the public health and environmental fields due to their very low infectious dose, their persistence in the environment (up to several years), and their high resistance to conventional disinfection processes. Helminthiasis is endemic in Africa, Latin America and East Asia where poverty and poor sanitary conditions prevail. Globally, around 2.5 billion people are affected by helminthiasis, leading to serious collateral effects on quality of life that result in diarrhea, severe problems of undernourishment, anemia and physical and mental under-development, mostly in children between 5 and 15 years of age (WHO 2006, 2012a, 2012b; Jiménez et al. 2016).

Helminth eggs are composed of layers with different physical and chemical characteristics that act as protection barriers to various environmental conditions and explain the high resistance of these organisms to inactivation (Wharton 1980; Fairweather & Threadgold 1981; Lysak et al. 1985; Alouini & Jemli 2001; Mahmoud & el-Alfy 2005; Quilès et al. 2006; Sengupta et al. 2011). Helminth eggs are therefore among the most important biological pollutants in environmental engineering as they represent a significant health risk associated with poor sanitation, the use of polluted water for irrigation and the disposal of excreta or sludge to land (Jiménez et al. 2007).
The possibility of disease transmission depends upon the viability and infectivity of pathogens, referring to their ability to develop until they reach the infectious stage (Horák 1994). Different treatment methods of inactivation of helminth eggs have been evaluated; these methods include processes of altering temperature, dryness or pH or the addition of chemicals such as peracetic acid (Maya et al. 2005). In addition, a very important indicator in the evaluation of the efficiency in all these treatment methods is measurement of viability.

In this context, the incubation test is the only standard procedure available for quantification (EPA 2003). This test requires 3–4 weeks of incubation until the larvae develop. Consequently, different methods to determine viability have been proposed to reduce the time required for analysis. These include the addition of dyes and molecular techniques (polymerase chain reaction) (Rocha et al. 2006).

Because of the long time periods required for determining not only viability but also the capacity for infection of helminth eggs (infectivity) using incubation methods, the aim of this work was to determine the optimal and minimum times required to quantify the inactivation of the eggs of two species from two nematode genera (Ascaris lumbricoides and Toxocara canis) by the incubation process, using an automatic recognition tool.

**BACKGROUND**

**Helminth eggs, biological pollutants in environmental engineering**

The use of domestic wastewater for irrigation and the disposal of sludge, often without prior treatment, was common practices in developing countries. They lead to the proliferation of helminth eggs, the infective stage of the life cycle of the helminths, commonly known as intestinal worms. These are the most important biological pollutants in environmental engineering as they represent a significant health risk associated with poor sanitation (WHO 2006). This risk is linked to their infectiousness and resistance. In particular, this applies to the genus Ascaris, considered the most resistant form and the most difficult biological parasite to inactive in wastewater and sludge (Alouini & Jemli 2001; WHO 2006) due to its low infectious dose, its persistence in the environment (up to several years), its high resistance to inactivation processes (chlorination, UV, ozone, etc.), and its high ovipositional capacity. Following a series of epidemiological studies, since 1989 the World Health Organization (WHO) has recommended limiting content to <1 viable helminth egg per litre of water (1 HE/L) and <1 viable egg per gram of total dry solids (1 HE/gTS) for sludge (WHO 1989, 2006, 2012a, 2012b).

**Wastewater characterization**

The content of helminth eggs in wastewater in developing countries varies between 70 and 3,000 HE/L, while for developed countries the content is much smaller, at concentrations of 1–9 HE/L (Jiménez & Wang 2006). After secondary treatment the number of helminth eggs may be reduced, but the number is generally greater than the limits established by the WHO (Table 1). Efficient inactivation processes are necessary, together with techniques that allow rapid and efficient evaluation of inactivation of eggs. This is of particular importance in the sanitary field.

**Viability and infectivity determination**

In the literature, several criteria have been reported to identify the viability of helminth eggs (Table 2). These generally define a viable egg as one that develops a specific structure and a non-viable egg as one that has a poorly defined structure.

In addition to viability, the infectivity (ability of the larva to cause an infection) is another factor of great importance; this can only be determined once the larva is fully developed, and has mobility and the ability to leave the egg on its own. As shown in Table 3, egg development depends on the incubation time, and the larva cannot be considered infective until day 18, when it has movement and can hatch.

When considering infectivity, some authors go further. For example, Johnson et al. (1998) administered helminth eggs orally to worm-free pigs, then recovered adult parasites from the small intestine at necropsy and observed damage to internal organs caused by migrating infective larvae. The authors used this as infective method analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw wastewater Average</th>
<th>Secondary effluent Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity, NTU</td>
<td>136</td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18.8</td>
<td>19.2</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD) (mg/L)</td>
<td>359</td>
<td>74</td>
</tr>
<tr>
<td>Total suspended solids (mg/L)</td>
<td>770</td>
<td>150</td>
</tr>
<tr>
<td>Helminth eggs (HE/L)</td>
<td>80</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 1 | Physicochemical and biological characterization of raw wastewater and treated wastewater (Jiménez et al. 1999, 2007)
Incubation test

As mentioned previously, to determine the viability and infectivity of the helminth eggs, the eggs are generally incubated at 28°C using a 0.1 N H2SO4 solution for 20 days. The US EPA method considers six stages of helminth egg development (egg, four larval stages, and adult) (EPA 2003). Ayres et al. (2013) reported nine stages of development: single cell, two cells, four cells, 4–16 cells, late morula-blastula, gastrula, tadpole, immature larva, and mature larva. Contrary to this, Cruz et al. (2012) identified 12 stages of development: on cell, two cells, three cells, four cells, early-morula, late-morula, blastula, gastrula, pre-larva 1, pre-larva 2, first-stage larva (L1), and second-stage larva (L2). Kim et al. (2012) mentions that the effect of temperature conditions, 25°C and 35°C, using a 0.1 N H2SO4 solution induced *Ascaris suum* egg development to the eight-cell stage at days 5–6.

**Inactivation temperature conditions**

In a study reported by Carrington et al. (1991) the exposure of *A. suum* to a temperature of 53°C for 1 h was sufficient to destroy its viability. Seamster (1950) reported that the application of a temperature of 37.8°C could cause the inactivation of helminth eggs after 8 days. This temperature was found to be the lower limit for inactivation. According to Maya et al. (2012), a temperature of 45°C for 6 days was sufficient to inactivate different genera of helminth eggs, where the most resistant was *Ascaris*.

**Method of incubation for viability determination**

Another interesting consideration for the conditions of incubation for viability determination is the concentration of the eggs in the suspension. Eriksen (1990) observed that the rate of development to the infective stage is very slow when egg concentration in a given suspension is high. In contrast, in solutions with a low concentration of eggs (25 eggs/μL), larvae developed within 4 weeks. No larval development was observed for extremely high egg concentrations (100 eggs/μL).

Table 4 summarizes the conditions of incubation used in the literature for the development of the larva. This review suggests that there is a lack of uniformity in the techniques of such tests, which is reflected in the results (WHO 2004).

**METHODOLOGY**

The effects of temperature on inactivation were tested on species from two nematode genera: *Ascaris lumbricoides* and *Toxocara canis*. Both genera have been reported in wastewater, excreta, and sludge of different countries (Kozan et al. 2005; Jiménez & Wang 2006; Sidhu & Toze 2008).

**Seeded samples and wastewater preparation**

Helminth eggs of *Ascaris lumbricoides* and *Toxocara canis* were obtained from certified suspensions of 20,000 eggs/mL of each species, purchased from the Pathology Department.
of the Biology Institute of the National Polytechnic Institute (Mexico City). Subsequently, a series of steps of extraction, filtration with 20 μm sieve, and centrifugation at 600 g in an IEC centrifuge (model HN-SII) were carried out to obtain a concentrated suspension containing 50 eggs/50 μL of each species (Maya et al. 2012). For each experiment, samples with a mixture of 100 helminth eggs (50 of each of the two species) were inoculated to a volume of 50 mL of secondary effluent of wastewater sampled from the settling tank of a municipal wastewater treatment plant operating with a physicochemical process (150 gTS). The wastewater sample was filtered using a 38 μm sieve prior to inoculation to ensure it was free of Ascaris and Toxocara eggs, since their diameter is greater than 38 μm.

**Thermal inactivation process**

Ascaris lumbricoides and Toxocara canis egg suspensions were exposed to a temperature of 60 °C for 1 h, in a Cole-Parmer water bath (model 12501-00). Once the contact time was completed for each concentrate, helminth eggs were recovered by filtration using a 20 μm pore diameter and centrifugation at 600 g. A quantification stage was performed to evaluate how many viable helminth eggs remained in the sample. A third helminth egg suspension containing eggs of both species was not subjected to heat and was used as a control. Each study was carried out in triplicate.

**Viability assessment – incubation test**

To quantify and determine inactivation of nematode eggs, the three suspensions were incubated at 28 °C and 34 °C for 20 days in a 0.1 N solution of sulfuric acid. Subsequently, they were washed with distilled water, filtered with a 20 μm pore diameter sieve and then centrifuged at 600 g (Maya et al. 2012). Identification and quantification was carried out at 2, 4, 8, 16, 18, and 20 days by direct observation in

<table>
<thead>
<tr>
<th>Species</th>
<th>Incubation time (days)</th>
<th>Incubation temperature (°C)</th>
<th>Incubation solution</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris suum</td>
<td>18</td>
<td>28</td>
<td>0.1 N H₂SO₄</td>
<td>Incubation carried out in darkness</td>
<td>Cruz et al. (2012)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>56</td>
<td>Saline containing 0.8% formaldehyde</td>
<td></td>
<td></td>
<td>Horák (1994)</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>28</td>
<td>30</td>
<td>0.1 N H₂SO₄</td>
<td>Under optimum conditions, all viable eggs become fully embryonated after 21–28 days</td>
<td>Ayres et al. (1995)</td>
</tr>
<tr>
<td>Ascaris, Toxocara, Capillaria and Trichuris</td>
<td>21</td>
<td>26</td>
<td>0.1 N H₂SO₄</td>
<td></td>
<td>Mignotte-Cadiergues et al. (2001)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>28</td>
<td>27</td>
<td>0.1 N H₂SO₄</td>
<td></td>
<td>Gaasenbeek &amp; Borgsteede (1998)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>20</td>
<td>30</td>
<td>0.1 N H₂SO₄</td>
<td></td>
<td>Plym-Forshell (1995)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>90</td>
<td>22</td>
<td>0.1 N H₂SO₄</td>
<td>7, 14, 21, 40, 60 and 90 days</td>
<td>Ghiglietti et al. (1997)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>28</td>
<td>28</td>
<td>0.1 N H₂SO₄</td>
<td>When incubated at 30 °C, Ascaris eggs develop into vermiform motile embryos after 9 days</td>
<td>Jones &amp; James (1985)</td>
</tr>
<tr>
<td>Ascaris spp.</td>
<td>20</td>
<td>30</td>
<td>0.1 N H₂SO₄</td>
<td></td>
<td>Kaulenas &amp; Fairbairn (1966)</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>21</td>
<td>26</td>
<td>Physiological saline with two droplets of K₂Cr₂O₇</td>
<td></td>
<td>Plachý et al. (1993)</td>
</tr>
<tr>
<td>Helminth eggs</td>
<td>20</td>
<td>26</td>
<td>0.1 N H₂SO₄</td>
<td></td>
<td>Maya et al. (2012)</td>
</tr>
<tr>
<td>Nematoda eggs</td>
<td>20</td>
<td>26–28</td>
<td>0.1 N H₂SO₄</td>
<td>Incubation with oxygenation</td>
<td>Jiménez et al. (2002)</td>
</tr>
</tbody>
</table>
a Sedgewick Rafter chamber with 50 × 20 mm dimensions and a 1 mm depth (Thomas Scientific, Swedesboro, New Jersey, USA), under 10× magnification with a Carl Zeiss XA optical microscope. It was then determined whether the eggs showed internal structural development. The criteria for determination of helminth egg viability, listed in Table 2, were used. Those that did not present two or more clearly defined cells after 48 h were considered inactivated. Those containing two or more clearly defined cells after 48 h were re-incubated until the motile larval stage to confirm their complete inactivation. Presence of mobile larvae was considered a confirmation of viability, while hatching demonstrated infectivity. Larval movement could be detected within 5–10 min observation under the microscope.

**Staining with Trypan Blue**

To determine the efficiency of a Trypan Blue staining process, after 20 days of incubation a suspension of 50 *Ascaris* and 50 *Toxocara* viable eggs that were not subjected to inactivation was dyed with 10 mL of a solution of 0.1% Trypan Blue and examined under 10× and 63× magnification with a Carl Zeiss XA optical microscope.

**Viability assessment – automatic system**

The Engineering Institute of the National Autonomous University of Mexico (UNAM) has been developing the Helminth Egg Automatic Detector (HEAD), a system that can identify and quantify pathogenic species of helminth eggs. It analyzes a microscopic digital image using a set of digital filters that extract the morphology, egg gray level statistics and texture of the helminth eggs to later classify them (Jiménez et al. 2016). The system has the possibility of integrating the ability to detect the presence of the egg nucleus and even verify whether the same nucleus has divided or not after 48 h under various incubation conditions. This allows confirmation of viability of the egg or its inactivation.

The main characteristics that allow the HEAD system to differentiate division of the nuclei of eggs of *Ascaris lumbricoides* and *Toxocara canis* during the incubation process are the Gray Level, Kurtosis, and Entropy of the Gray Level. These characteristics are described below:

The Standard Deviation of the Gray Level is a statistical value that represents the uniformity of the behavior of the gray level pixels within the core of the egg. The pixel is the smallest part of a digital image and the gray level refers to the pixel value after changing a color image to a gray scale. This value is calculated using the following equation:

\[
S = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (np_i - \mu)^2}
\]

where:
- \( S \) is standard deviation
- \( N \) is the number of pixels that form the egg nucleus
- \( \mu \) is nucleus mean gray level value
- \( np \) is a nucleus pixel.

Kurtosis is the gray level statistic and the measure of how outlier-prone is the pixel distribution of the nucleus. Thus it describes the behavior of the pixels within that region. It is a vital characteristic since it allows differentiation between a nucleus with or without divisions.

It is calculated using the following equation:

\[
K = \frac{E(x - \mu)^4}{\sigma^4}
\]

where:
- \( K \) is kurtosis
- \( \mu \) is nucleus mean gray level value
- \( \sigma \) is nucleus standard deviation of the gray level values
- \( E(t) \) represents the expected value of the quantity \( t \)
- \( x \) is set off nucleus pixels' gray values.

Entropy of the Gray Level values is a statistical measure of the randomness presented in the nucleus region; it is used as a basic texture characteristic descriptor. The following equation represents how it is calculated:

\[
E = - \sum_{i=1}^{N} p_i \times \log (p_i)
\]

where:
- \( E \) is entropy of the nucleus
- \( N \) is the number of pixels that form the egg nucleus
- \( p \) contains the normalized histogram counts.

**RESULTS AND DISCUSSION**

**Viability assessment – incubation test**

Viability and infectivity (by hatching) of suspensions of *Ascaris lumbricoides* and *Toxocara canis* eggs can be
observed in Figure 1. After incubation at both temperatures, 28 °C and 34 °C, the development of the egg occurred at the same rate. It has not been observed that an increase in temperature reduces the required incubation time. Arene (1986), for example, mentions that an increase in temperature of 30–34 °C accelerates larval development in the case of *A. suum* eggs; however, when comparing the results with the present study, the increase in temperature did not imply an actual reduction of the incubation time to determine viability, much less the infectivity, which occurs between 18 and 20 days of incubation.

*Kim et al. (2012)* reported that temperatures of 25 °C and 35 °C induced egg development to the eight-cell stage at days 5–6 after incubation. The formation of larvae at temperatures of 35 °C and 25 °C occurred at 17 and 19 days after incubation, respectively. However, an increase in incubation temperature may have an adverse effect on egg development. This is corroborated by Seamster (1950) who reported that the application of a temperature of 37.8 °C could cause the inactivation of helminth eggs after 8 days. This becomes an important factor in the evaluation of inactivation processes since temperature could also act as a parameter that contributes to the inactivation process.

Table 5 shows that on the second day of incubation 100% of *Ascaris lumbricoides* and *Toxocara canis* eggs exposed to a temperature of 60 °C for 1 h in all suspensions were inactivated. It is therefore unnecessary for them to undergo incubation for 20 days, since the samples no longer show changes in the inactivation percentage and there is evidence that 48 hours is sufficient time to determine inactivation.

According to the methodology developed by *Johnson et al. (1998)*, after an incubation period of 48 h (28–34 °C) samples of helminth eggs should be examined under the optical microscope. Non-viable eggs are considered to be those that do not present internal structural development while viable eggs are those that contain two or more clearly defined cells. Viable eggs should be incubated again until the motile larval stage is reached, whereby it is determined that the egg is not only viable, but also infective. Furthermore, *Kim et al. (2012)* reported that eggs cultivated in an environmental chamber at 25 °C also presented two-cell development on the second day of incubation.

In this context, and according to the literature, the time required for analysis of viability could be reduced to 2 days, which would mean, in the case of studies to evaluate inactivation processes, results may be obtained much more rapidly. Another important aspect, besides the ability of the egg to develop into a larva, is its infectivity, which can be determined once the larva reaches its maximum development; this is achieved on day 18 of incubation, since, from this point onwards, the L2 larva has mobility and is capable of hatching, a characteristic which cannot be determined in previous stages. Larval movement may be detected within 5–10 min observation under the microscope.

**Staining with Trypan Blue**

After 20 days of incubation, the control suspensions of 50 viable eggs of *Ascaris* and 50 viable eggs *Toxocara* that were not subjected to inactivation were dyed with a solution
of 0.1% Trypan Blue. This showed that: (a) 26% of Ascaris and 43% of Toxocara eggs in the control sample were dyed.

Even though dyes may reduce the time for assessing viability of helminth eggs to approximately 3–5 minutes (Jones & James 1985; Horák 1994), the use of Trypan Blue dye may lead to an underestimation of viability by almost 50% (Karkashan et al. 2015).

Figure 2 shows the presence of infective larvae in Toxocara canis eggs dyed with Trypan Blue. In theory, those eggs that become dyed should be non-viable, and yet the hatching of a viable and infective mobile larva may be observed. It is clear that the dyeing process is neither efficient nor practicable and it is therefore preferable to use an incubation process despite it taking much longer (20 days).

Viability assessment – automatic system

The HEAD system for the identification and quantification of different species of helminth eggs, including Ascaris lumbricoides and Toxocara canis (Jiménez et al. 2016), represents a quick alternative. It involves training a neural network classifier, and uses the pixel Gray Level and texture characteristics discussed previously (Standard Deviation, Kurtosis, and Entropy).

The HEAD system was used, implementing the methodology proposed by Lozano et al. (2005), to detect and quantify cell micronucleus configurations due to DNA patterns. It was the basis to be able to verify more clearly if the egg was viable or not (Figure 3).

In the different tests developed, it was possible to observe differences in the characteristics related to the gray level between the eggs that presented cell division and those that did not (Tables 6 and 7).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Quantification of percentage inactivation by thermophilic inactivation process at 60 °C for 1 h, after incubation at 28 °C and 34 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of incubation (days)</td>
<td>Number of eggs inactivated (28 °C)</td>
</tr>
<tr>
<td>Helminth egg species</td>
<td>Triplicate</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Toxocara canis</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

All the solutions contained 100 eggs. All determinations were made in triplicate. *Egg(s) lost in one of the recovery stages during the determination of inactivation.

Figure 2 | Hatching of Toxocara control dyed with Trypan Blue after incubation in 0.1 N H2SO4 at 28 °C by optical microscopy observation. Photograph: archive of the Treatment and Reuse Group, II-UNAM.

Figure 3 | Preliminary results for the detection of the egg nucleus to verify if there is cellular division.
In addition, as shown in Tables 6 and 7, for Ascaris eggs the standard deviation is larger for the eggs where the nucleus is undergoing division, making it clear that there is no uniformity. Another value that confirms lower uniformity in the egg nuclei is the Entropy, for which the average value is slightly larger in comparison with the eggs not undergoing division. The Kurtosis values are bigger in the eggs without division; this means that the Gray Level pixel values are nearer to their statistical mean, confirming the uniformity of the structure. A similar behavior can be observed in the Toxocara eggs: larger Standard Deviation with lower Kurtosis within the nucleus zone. However, the Entropy was larger for the eggs without division. This can be explained by the fact that the Standard Deviation is bigger than the mean value, introducing uncertainty to this preliminary result.

CONCLUSIONS

The health risks of reuse of wastewater in agricultural practices are related to the helminth eggs they may contain. In guidelines these eggs are considered to be the biological particles of greatest resistance to treatment, and under environmental conditions they remain viable for several months or even years. To resolve this risk, different processes of inactivation have been applied worldwide in order to comply with the established limits of 1 non-viable egg/L. However, to evaluate the efficiency of these different processes, efficient and fast methodologies are required to determine whether eggs have been inactivated or continue to be viable.

One of the evaluation methods available is the incubation method, whose disadvantage is the long period of time required to determine the viability and infectivity of helminth eggs. The objective of the present work was to determine the optimal and minimum time required to show the inactivation of eggs of the helminth species Ascaris lumbricoides and Toxocara canis.

An inactivation process was applied to a suspension containing a known number of eggs of A. lumbricoides and T. canis. After incubation at a temperature of 60 °C for 1 h it was possible to determine inactivation due to the absence of nucleic division at 48 hours. This indicates that the egg is no longer viable, which was fully confirmed by 20 days of incubation. The efficiency was 100%.

Simultaneously, using control suspensions of viable eggs that were not subjected to inactivation, and after incubation for 20 days, the efficiency of the Trypan Blue staining technique was observed. The results indicated that it is not entirely efficient, and even less so when used to determine the infectivity of an egg in order to comply with the regulations for unrestricted irrigation. The percentage of the dyed, presumably non-viable, eggs was 26% for Ascaris and 43% for Toxocara.

Another alternative to evidence inactivation of helminth eggs is the automatic HEAD system, used to detect division of the nucleus after 48 h of incubation. The values of Gray Level, Kurtosis and Entropy were used to train a multilayer perceptron neuronal network, through which it may be determined automatically if the eggs present cell division. This may be used in future to assess the efficiency of inactivation processes, specifically for the species Ascaris lumbricoides and Toxocara canis.

ACKNOWLEDGEMENTS

This study was carried out with support from the Bill and Melinda Gates Foundation (Grant ID # OPP1149050).

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First received 23 September 2019; accepted in revised form 30 January 2020. Available online 13 February 2020.