Food and Waterborne Parasitology 3 (2016) 9–12

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

journal homepage: www.elsevier.com/locate/fawpar

An approach to reduce false viability assessment of hookworm eggs with vital stains

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ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form 21 February 2016
Accepted 3 March 2016
Available online 10 March 2016

Keywords:
Hookworm eggs
Viability assessment
Vital stains
Public health
Wastewater

ABSTRACT

The effectiveness of vital stains to assess the viability of hookworm eggs depends on the permeability of eggshells. The eggshells may not be permeable immediately after inactivation, and this can lead to over-estimation of viable eggs in a sample. In this study, heat-inactivated eggs of hookworm (*Ancylostoma caninum*) were used to evaluate the time required for eggshells to become permeable to three vital stains (eosin Y, methyl red, and methylene blue). The results of this study showed that heat-inactivated eggs incubated for 1 h at room temperature (24 ± 1 °C) contain 32–53% non-viable eggs or permeable eggshells. The numbers of non-viable eggs increased to 88–94% after 6 h of incubation. The accurate viability assessment was achieved after 12 h of incubation at room temperature. Vital stains did not play a significant ($P > 0.05$) role in reducing the false viability. This study provides information that can aid in the accurate detection of viable hookworm eggs from environmental samples comprised of mixed populations that are viable and non-viable with permeable or impermeable eggshells.

1. Introduction

Hookworm infection is responsible for malnutrition, anemia, and impaired cognitive development in humans (Brooker et al., 2008; WHO, 2012). It is estimated that approximately 8.0×10^6 people worldwide are infected with hookworm (Hotez et al., 2014). Despite implementation of several interventions to reduce hookworm infection in developing countries, it is difficult to control (WHO, 2012; WHO, 2015). Land application of untreated wastewater and sludge is a common practice in developing countries (Do et al., 2007; Gupta et al., 2009). As a result, a high load of viable hookworm eggs can be present in the environment, which may pose a significant human health risk (Karkashan et al., 2014).

Several guidelines have been developed by national and international authorities to minimize the risk of hookworm infection associated with wastewater and sludge (US EPA, 2003; NRMMC, 2004; WHO, 2006). These guidelines, however, do not provide a standardized protocol for reliable detection and quantification of viable hookworm eggs from wastewater matrices. It is necessary to detect viable eggs in order to determine accurate health risk if wastewater is used for land application. Conventional egg viability assessment method such as incubation is time consuming, expensive and laborious (Karkashna et al., 2014; Gyawali et al., 2015).

Vital stain method provides information on the viability of hookworm eggs more rapidly than the incubation method (Dabrowaska et al., 2014; Karkashan et al., 2014). Vital stain method takes advantage of structural integrity of viable and non-viable eggshells. A viable egg has three layers of intact shells that act as an alternative barrier and prevent stain from entering into the cytoplasm (Matthews, 1986). Once the egg becomes non-viable, the integrity of eggshells is compromised and becomes

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http://dx.doi.org/10.1016/j.fawpar.2016.03.001

permeable to stains (Bae and Wuertz, 2009). The eggshells, however, may not be permeable to vital stains immediately after inactivation. In this study, we evaluated time required for accurate viability assessment of hookworm eggs using three commonly used vital stains. These stains include eosin Y (MW = 647.8), methyl red (MW = 269.3), and methylene blue (MW = 319.8). They were chosen to evaluate, whether molecular weight of a particular stain can influence the assessment of viability.

2. Material and methods

Dog fecal samples were collected after obtaining ethics approval from the Animal Ethics Committee, The University of Queensland, Australia (Reference number: AEC/QU/12/2013). Eggs of the dog hookworm [*Ancylostoma caninum*] were isolated from the fecal

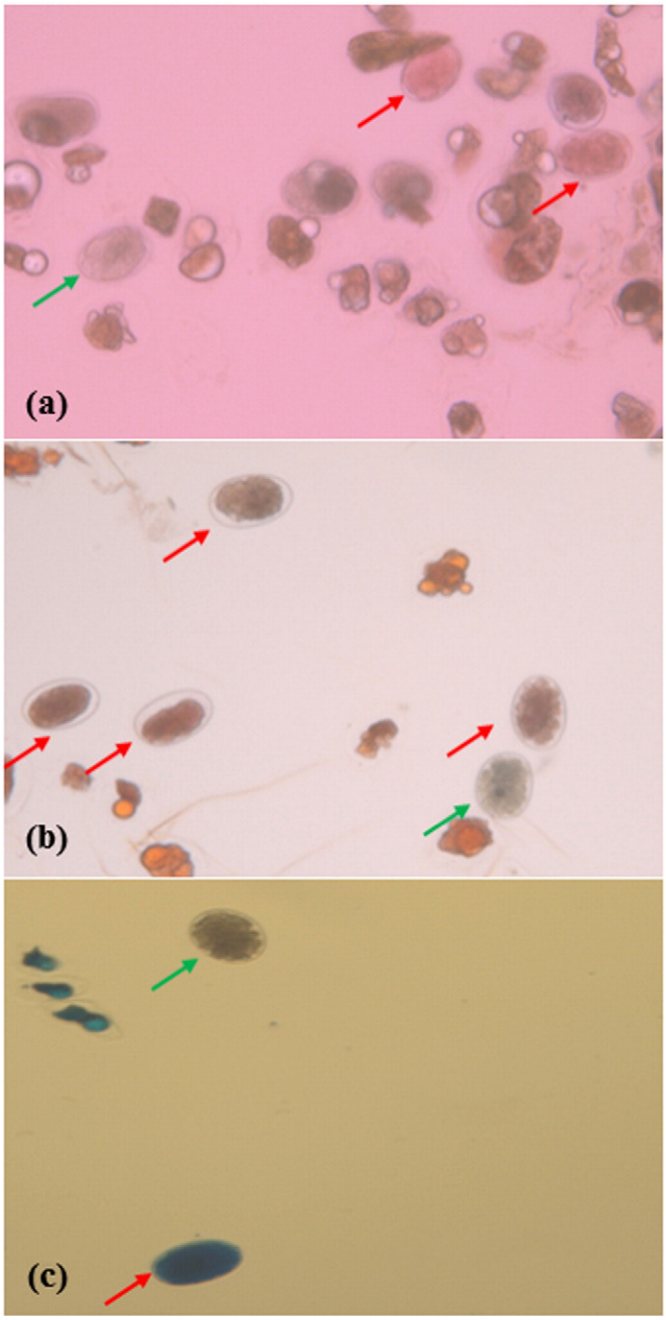

Fig. 1. Photomicrographs (100×) of viable (green arrow) and non-viable (red arrow) *Ancylostoma caninum* eggs by different stains (a) eosin Y, (b) methyl red and (c) methylene blue.