Infectious helminth ova in wastewater and sludge:  
A review on public health issues and current 
quantification practices  

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ABSTRACT

Raw and partially treated wastewater has been widely used to maintain the global water demand. Presence of viable helminth ova and larvae in the wastewater raised significant public health concern especially when used for agriculture and aquaculture. Depending on the prevalence of helminth infections in communities, up to $1.0 \times 10^3$ ova/larvae can be presented per litre of wastewater and 4 gm (dry weight) of sludge. Multi-barrier approaches including pathogen reduction, risk assessment, and exposure reduction have been suggested by health regulators to minimise the potential health risk. However, with a lack of a sensitive and specific method for the quantitative detection of viable helminth ova from wastewater, an accurate health risk assessment is difficult to achieve. As a result, helminth infections are difficult to control from the communities despite two decades of global effort (mass drug administration). Molecular methods can be more sensitive and specific than currently adapted culture-based and vital stain methods. The molecular methods, however, required more and thorough investigation for its ability with accurate quantification of viable helminth ova/larvae from wastewater and sludge samples. Understanding different cell stages and corresponding gene copy numbers is pivotal for accurate quantification of helminth ova/larvae in wastewater samples. Identifying specific genetic markers including protein, lipid, and metabolites using multiomics approach could be utilized for cheap, rapid, sensitive, specific and point of care detection tools for helminth ova and larva in the wastewater.

Key words | detection methods, helminth ova, public health risk, sludge, wastewater reuse

INTRODUCTION

Freshwater resources are under pressure through climate change and increasing population with high demand of fresh water. In this situation, wastewater can be used as an alternative source of water to reduce the pressure on non-potable demand of freshwater. One of the most significant issues in relation to wastewater reuse is the potential public health risk associated with viable helminth ova/larva (Jimenez et al. 2007). Therefore, treatment is a must before using wastewater for non-potable purpose where there is potential for human exposure (US EPA 2005; NRMMC 2004; WHO 2006).

Due to the high level of nutrients in raw and partially treated wastewater, it has been widely used for food production such as agriculture and aquaculture, especially in the developing countries (Carr 2005; Toze 2006a; Vuong et al. 2007; Sidhu & Toze 2009; Pinto & Maheshwari 2010; Pritchard et al. 2010; Hanjra et al. 2012). Despite the reuse of wastewater being mainly associated with socio-economic factors such as poverty and awareness of co-existed health risks, the global trend of wastewater and sludge reuse for crop production has increased significantly in the last decade (Oleszkiewicz & Mavinic 2001; Jimenez 2006; Sidhu & Toze 2009; Pinto & Maheshwari 2010; Pritchard et al. 2010; Hanjra et al. 2012; Kelessidis & Stasinakis 2012).

Infected individuals release helminth ova/larvae into wastewater that can potentially contaminate soil, plant, and surface water depending on the wastewater reuse. In those environments, helminth ova can survive up to several years (Sanguinetti et al. 2005; Abaidoo et al. 2010). For example, eggs of Ascaris lumbricoides can survive up to...
15 years in an environment upon favourable conditions (Hagel & Giusti 2010). Exposure to the wastewater and sludge or soil and crops contaminated with the viable helminth ova/larvae present in the environment, infective dose, exposure routes and the susceptibility of the exposed individual (Haas 1996; Haas et al. 1999; Navarro & Jimenez 2011).

Since a viable ovum/larva has the potential to cause infection in humans (WHO 2006; Toze 2006b), a thorough understanding of the prevalence of viable helminth ova/larvae in wastewater and sludge is essential to understand the infection potential, and the risks they pose from beneficial reuse. The main aims of this literature review are (i) to determine the prevalence of helminths ova in wastewater and sludge, (ii) overview of public health risk, and (iii) critically examine the currently available quantification methods. Information presented in this paper will encourage researchers to look for a new detection method that can accurately quantify viable helminth ova from wastewater and sludge.

Articles, reports, conference proceedings, and guidelines published from 2000 to 2016 were taken into consideration. Electronic databases including PubMed, Google Scholar, and Web of Knowledge were used to obtain the information. The literature search was performed using keywords (helminth ova in wastewater, helminth ova in sludge, wastewater treatment methods and helminth ova inactivation, sludge treatment methods and helminth ova inactivation). Since *Ascaris lumbricoides* is being used as an indicator organism, search criteria for literature were broadened using keywords (*Ascaris* in wastewater, *Ascaris* in sludge, wastewater treatment and *Ascaris* ova inactivation). Overall, 178 items of literature were reviewed and information from 134 were used to prepare this article.

**PREVALENCE AND CONCENTRATION OF HELMINTH OVA AND LARVAE IN WASTEWATER AND SLUDGE**

The occurrence of helminth ova/larvae in wastewater and sludge depends on the prevalence of infections in the surrounding communities (Gaspard & Schwartzbrod 2003; Hajjami et al. 2012; Sharafi et al. 2012; Bastos et al. 2013). Soil-transmitted helminths (STHs) (*Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus,* and *Trichuris trichura*) are detected more often than others. This is due to the fact that i) STHs are the most prevalent parasites around the world with an infection rate of $1.3 \times 10^9$; ii) a mature STH female can produce large numbers of ova each day (Bethony et al. 2006; Hotez et al. 2014; WHO 2015) (Table 1); and iii) *A. lumbricoides* has been used as a model parasite to determine the quality of treated wastewater and sludge. However, ova/larvae of other helminths such as *Enterobius vermicularis, Strongyloides stercoralis, Toxocara spp., Taenia spp., Hymenolepis nana, Echinococcus spp., Trichostrongylus spp.*, and *Dicrocoelium dendriticum* have also been found in wastewater and sludge samples (Gaspard & Schwartzbrod 2003; Mahvi & Kia 2006; Do et al. 2007; Jimenez et al. 2007; Wichuk & McCartney 2007; Ben Ayed et al. 2009; Hajjami et al. 2012; Sharafi et al. 2012; Bastos et al. 2013; Konate et al. 2013a, 2013b).

Due to the lack of a robust and uniform method, for isolation and quantification of helminth ova from wastewater and sludge, it is almost impossible to make an accurate comparative assessment of the concentration of ova in wastewater and sludge samples within and between the studies (Amoah et al. 2017). The concentration of ova in raw wastewater (1 L) and sludge (4 gm dry weight) can be as high as $10^3–10^6$ depending upon the rate of infection in the community (Table 2). Due to the high settling velocity of helminth ova, their concentration in sludge should be higher than wastewater (Konate et al. 2010; Sengupta et al. 2011, 2012). However, Navarro & Jimenez (2011) reported high concentration of helminth ova in wastewater compared to the sludge samples. This could be due to poor recovery rate of helminth ova from sludge compared to the wastewater samples using flotation method. Gyawali et al. (2015a) reported poor (0.02–3.4%) recovery rate of helminth ova from sludge samples compared to the wastewater (7.1–7.4%).

Climatic conditions such as temperature and relative humidity can also influence the numbers of ova and larvae in the wastewater and sludge (Hajjami et al. 2012; Sharafi et al. 2012; Bastos et al. 2013). This is because helminth ova are known to develop faster at a temperature between 28 and 32 °C (Seamster 1950; Beer 1976; Smith & Schad 1989; Brooker et al. 2006). In addition, soil moisture and relative humidity also influence the survival of viable ova and larvae (Nwosu & Anya 1980; Udonsi et al. 1980; Brooker et al. 2006). Therefore, this potentially infects the wider population which eventually contributes more ova into the wastewater system.

**POTENTIAL PUBLIC HEALTH RISKS**

Wastewater and sludge contaminated with helminth ova and larvae can pose significant public health risks. People become
People also acquire helminth infections via indirectly exposure to helminth ova/larvae present in the wastewater and sludge. For example, handling and consuming products (vegetable and aquaculture) grown on farms that use wastewater and sludge (Ensink et al. 2008; Seidu et al. 2008; Ackerson & Awuah 2012). Health risk among this group (wastewater workers and farmers using partially treated wastewater) is associated with their working conditions, individual behaviour, personal hygiene and use of personal protective equipment (El Kettani et al. 2011; The World Bank 2010). The health risk in this group is significantly higher in developing countries compared to developed countries (Ensink et al. 2005; El Kettani & Azzouzi El 2006; Trang et al. 2006; The World Bank 2010), due to i) the higher prevalence rate of helminth infections in developing countries compared to the develop countries; ii) poverty; iii) lack of health education regarding the potential transmission of helminth; and iv) lack of use of personal protective equipment.

People also acquire helminth infections via indirectly exposure to helminth ova/larvae present in the wastewater and sludge. For example, handling and consuming products (vegetable and aquaculture) grown on farms that use wastewater and sludge (Ensink et al. 2005; Vuong et al. 2007; Gupta et al. 2009; Al-Megrm 2010; Iwamoto et al. 2010; Navarro & Jimenez 2011; Ackerson & Awuah 2012; Adamu et al. 2012). Helminth ova contained in the wastewater (treated or raw) and sludge can contaminate both surface (e.g. spinach and parsley) and underground (e.g. carrots and radish) crops (Navarro et al. 2009; Navarro & Jimenez 2011; Rostami et al. 2016). It has been reported that up to 90% of helminths ova present in the irrigated water can get onto green and leafy vegetables such as spinach and parsley with magnitude of 1 ovum/10 gm vegetable (Amahmid et al. 1999; Vuong et al. 2007; Navarro & Jimenez 2011). The magnitude of the contamination in subsurface vegetables, however, is much (98%) lower than in leafy vegetables (Amahmid et al. 1999). This is because the leafy vegetables like spinach, lettuce and cabbage have large contact areas for STHs ova/larvae. In addition, the magnitude of the contamination increases in the rainy season (Vuong et al. 2007) due to either flooding or shifting helminth ova/larvae from the ground to the vegetables by rain. Similarly, helminth ova present in the wastewater could accumulate in fish or shellfish through a feeding mechanism where they survive until finding the human host. The potential health risks to this groups can increase with the growing trend of eating raw vegetables and seafood for the sake of nutrients. Additionally, the international transportation of fruits, vegetables and seafood has increases the human health risks in global scale.

Gardens, parks and sporting venues that use wastewater for irrigation and waterways contaminated with wastewater runoff can contain viable helminth ova. Using those land or

### Table 1: Commonly detected helminths in wastewater and sludge, infective stage and transmission mode to humans

<table>
<thead>
<tr>
<th>Helminths</th>
<th>No of ova/larvae produced by a female</th>
<th>Infective stage</th>
<th>Transmission mode</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris</em> spp.</td>
<td>$2 \times 10^5$</td>
<td>Embryonated ova</td>
<td>OI</td>
<td>Knopp et al. (2012)</td>
</tr>
<tr>
<td>Hookworm</td>
<td>$3 \times 10^4$</td>
<td>Filariform larvae</td>
<td>OI/SP</td>
<td>Bethony et al. (2006)</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>$5 \times 10^5$</td>
<td>Embryonated ova</td>
<td>OI</td>
<td>Knopp et al. (2012)</td>
</tr>
<tr>
<td><em>E. vermicularis</em></td>
<td>$1.6 \times 10^4$</td>
<td>Embryonated ova</td>
<td>OI</td>
<td>Burkhart &amp; Burkhart (2005)</td>
</tr>
<tr>
<td><em>S. stercoralis</em></td>
<td>$4 \times 10^4$</td>
<td>Filariform larvae</td>
<td>SP</td>
<td>Ganesh &amp; Cruz (2011)</td>
</tr>
<tr>
<td><em>Toxocara</em> spp.</td>
<td>$2 \times 10^5$</td>
<td>Embryonated ova</td>
<td>OI</td>
<td>Azam et al. (2012)</td>
</tr>
<tr>
<td><em>Taenia</em> spp.</td>
<td>$5 \times 10^7$</td>
<td>Embryonated cyst</td>
<td>OI</td>
<td>Bogitsh et al. (2012)</td>
</tr>
<tr>
<td><em>Hymenolepis</em> spp.</td>
<td>NR</td>
<td>Embryonated cyst</td>
<td>OI</td>
<td>Bogitsh et al. (2012)</td>
</tr>
<tr>
<td><em>Echinococcus</em> spp.</td>
<td>NR</td>
<td>Embryonated ova</td>
<td>OI</td>
<td>Bogitsh et al. (2012)</td>
</tr>
<tr>
<td><em>D. dendriticum</em></td>
<td>NR</td>
<td>Metacercariae larvae</td>
<td>OI</td>
<td>Bogitsh et al. (2012)</td>
</tr>
<tr>
<td><em>Fasciola</em> spp.</td>
<td>$2.5 \times 10^4$</td>
<td>Metacercariae larvae</td>
<td>OI</td>
<td>Hanna et al. (2008)</td>
</tr>
<tr>
<td><em>Spirometra</em> spp.</td>
<td>NR</td>
<td>Coracidia larvae</td>
<td>OI</td>
<td>Bogitsh et al. (2012)</td>
</tr>
<tr>
<td><em>Schistosoma</em> spp.</td>
<td>$3 \times 10^2$</td>
<td>Metacercariae larvae</td>
<td>OI</td>
<td>Grevelding (2004)</td>
</tr>
</tbody>
</table>

*a* = day.

*b* = life time.

NR = not reported, OI = oral ingestion, SP = skin penetration.
waterways for recreational activities is another indirect way of human exposure to helminth ova/larvae present in wastewater (Horweg et al. 2006; Moubarrad & Assobhei 2007; King 2010). It has been demonstrated that school-aged children playing in a wastewater-irrigated park had an 18% higher rate helminths infection than those did not use the park (Moubarrad & Assobhei 2007). Pets, especially dogs, can carry helminth ova/larvae home from outside where there is helminth contamination (Gyawali et al. 2013), and thereby transmit to humans. This issue however, can be reduced by adopting good hygiene practices.

### Table 2 | Concentration of helminth ova in raw wastewater, treated wastewater and sludge in different locations

<table>
<thead>
<tr>
<th>Locations</th>
<th>Concentration of helminth ova in wastewater matrices</th>
<th>Sludge (ova/gm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>33&lt;sup&gt;As&lt;/sup&gt;, 11&lt;sup&gt;En&lt;/sup&gt;, 5&lt;sup&gt;St&lt;/sup&gt;, 4&lt;sup&gt;Ta&lt;/sup&gt;, 4&lt;sup&gt;Hy&lt;/sup&gt;, 2&lt;sup&gt;Ho&lt;/sup&gt;, 2&lt;sup&gt;Ty&lt;/sup&gt;</td>
<td>NR</td>
<td>Water Corporation (2012)</td>
</tr>
<tr>
<td>Brazil</td>
<td>33&lt;sup&gt;As&lt;/sup&gt;, 3&lt;sup&gt;En&lt;/sup&gt;, 3&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Cutolo et al. (2006)</td>
</tr>
<tr>
<td>Egypt</td>
<td>1&lt;sup&gt;As&lt;/sup&gt;</td>
<td>NR</td>
<td>Bastos et al. (2013)</td>
</tr>
<tr>
<td>France</td>
<td>33&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Bonatti &amp; Franco (2016)</td>
</tr>
<tr>
<td>Ghana</td>
<td>1&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Stott et al. (2003)</td>
</tr>
<tr>
<td>India</td>
<td>72&lt;sup&gt;As&lt;/sup&gt;, 15&lt;sup&gt;Ho&lt;/sup&gt;, 4&lt;sup&gt;Ty&lt;/sup&gt;</td>
<td>NR</td>
<td>Konate et al. (2010)</td>
</tr>
<tr>
<td>Iran</td>
<td>12&lt;sup&gt;As&lt;/sup&gt;, 5&lt;sup&gt;Ta&lt;/sup&gt;, 2&lt;sup&gt;Ho&lt;/sup&gt;, 1&lt;sup&gt;En&lt;/sup&gt;</td>
<td>NR</td>
<td>Stott et al. (2003)</td>
</tr>
<tr>
<td>Morocco</td>
<td>23&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Gantz et al. (2001)</td>
</tr>
<tr>
<td>Mexico</td>
<td>330&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>As NR H NR</td>
</tr>
<tr>
<td>Oman</td>
<td>177&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Plank et al. (2007)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>272&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Gupta et al. (2009)</td>
</tr>
<tr>
<td>Peru</td>
<td>10&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Tyagi et al. (2011)</td>
</tr>
<tr>
<td>Tunisia</td>
<td>960&lt;sup&gt;As&lt;/sup&gt;, 535&lt;sup&gt;En&lt;/sup&gt;, 208&lt;sup&gt;Ta&lt;/sup&gt;, 140&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Bina et al. (2004)</td>
</tr>
<tr>
<td>USA</td>
<td>8&lt;sup&gt;Hy&lt;/sup&gt;</td>
<td>NR</td>
<td>Mahvi &amp; Kia (2006)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>5730&lt;sup&gt;As&lt;/sup&gt;</td>
<td>NR</td>
<td>Koura et al. (2002)</td>
</tr>
</tbody>
</table>

The numbers present in the table is highest range reported in the study.

### PUBLIC HEALTH PREVENTION MEASURES

Various wastewater and sludge reuse guidelines have been established to minimise the public health risk associated with helminth ova/larvae present in wastewater (US EPA 2003; NRMMC 2004; WHO 2006; DEC 2012). The guidelines have identified the potential points for health risks and designed a multi-barrier approach (Figure 2).

This approach includes good safe agriculture practice, good manufacturing practice and good hygiene practices where wastewater and sludge treatment alone is not sufficient.
to prevent the potential public health risks (WHO 2006; IWMI and IWRC 2010). An acceptable limit of helminth ova in treated wastewater and sludge depending on their final use has been recommended on the basis of cost benefit analysis of wastewater treatment methods versus potential health risks. For example, World Health Organization (WHO) has purposed value of <1 viable ovum in 1 L of treated wastewater or 4 gm of dry sludge can be used without restriction.

Wastewater and sludge treatment process is the first and most important measure towards minimising the potential health risk associated with wastewater reuse. Various wastewater and sludge treatment methods have also been proposed to inactivate helminth ova from wastewater and sludge (US EPA 2003; WHO 2006). Since the specific gravity of STHs ova is higher than wastewater, they quickly settle down into wastewater (Sengupta et al. 2011, 2012). Therefore, simple wastewater treatment methods such as ponding also effectively (90–99%) remove helminth ova from liquid (Toze 2006b; Reinoso et al. 2011; Konate et al. 2013a) (Table 3). However, those methods will concentrate the ova in to the sludge (solid) where they can remain viable for a long period of time (up to 20 months) (Sanguinetti et al. 2005).

Different methods such as aerobic digestion, anaerobic digestion, lime stabilisation and heat treatment, depending on the availability of resources and feasibility, have been used to inactivate the helminth ova from sludge (Mendez et al. 2002; Bina et al. 2004; Capizzi-Banas et al. 2004; Mendez-Contreras et al. 2009; Maya et al. 2010; Reinoso

**Figure 1** | Pathway of helminth infection from wastewater and sludge reuse.

**Figure 2** | Flow chart showing multi-barrier approach to minimise the public health risk associated with wastewater and sludge reuse.
The potential public health risks associated with wastewater and sludge reuse is measured by quantifying the numbers of viable *A. lumbricoides* ova (US EPA 2005; WHO 2006). This approach may not represent the true health risk associated with wastewater and sludge reuse and needs to be changed, because types of helminths and their numbers in wastewater and sludge depend on the prevalence rate of infections in the community that generates the wastewater. Another fundamental issue related to the health risk measurement is the quantification method that is used to identify and quantitate the viable helminth ova from wastewater. Despite the advancement in quantitative detection methods, microscope dependent methods, i.e. culture-based (US EPA 2005) and vital staining (de Victorica & Galvan 2003) are being used to quantify viable helminth ova from wastewater and sludge samples.

**PUBLIC HEALTH RISK MEASUREMENT METHODS**

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Culture-based method

The culture-based method involves artificially hatching the ova in a laboratory. Helminth ova are incubated at 28–30°C for up to 28 days depending on the helminth, to allow the viable ova to hatch and are observed microscopically (Bowman et al. 2005) (Figure 3). Health regulators, including the United States Environmental Protection Agency (US EPA) and WHO, recommend this method because it has the ability to estimate the viability of helminth ova recovered from treated wastewater and sludge. The requirement of highly skilled personnel to accurately distinguish between ova/larvae of different helminths is a major disadvantage of this method (Traub et al. 2007; Verweij et al. 2007). In addition, the detection limit of the method depends on the detection sensitivity of a microscope that may not be sensitive enough to detect low numbers of larvae in a sample (Weber et al. 1991). Gyawali et al. (2017b) has reported that there is a loss of 33% of viable ova/larvae during visualisation. The most important limitation of the culture-based method is the lengthy wait for the result to be available which potentially increases the operational cost of the method. The main advantages and disadvantages of the culture-based method are listed in Table 4.

Vital stain method

The stain-based method is rapid, cheap and easy to use compared to the culture-based method (de Victorica & Galvan 2005). This method involves staining helminth ova with a vital stain such as Trypan blue, Congo red, Eosin Y, Hematoxylin, Methyl green, Safranin O, Methylene blue or Lugol’s iodine and counting the ova under a microscope. This method takes advantage of different working mechanisms of the cell wall of viable and non-viable ova. A viable helminth ovum has three layers of intact cell walls that act as an alternative sieve and prevent the stain from entering into the cytoplasm (Matthews 1986). Once the ovum becomes non-viable, the integrity of cell wall is compromised and it becomes permeable to stain (Bae & Wuertz 2009) (Figure 3). The cell wall, however, is not permeable immediately after inactivation, and this can lead to over-estimation of viable ova in a sample (Gyawali et al. 2016c).

Another major disadvantage of the vital stain method is the potential loss of significant (25%) numbers of ova during visualisation (Gyawali et al. 2017b). This might be associated with either an inbuilt error of a microscope or an individual’s error while quantifying helminth ova using the microscope (Table 4). To ease the individual error and over-estimation of the viability of the vital stain method, BacLight LIVE/DEAD staining method has been developed (Dabrowska et al. 2014; Karkashan et al. 2015). Using fluorescent microscopy and membrane permeable DNA-labelling dyes, such as Syto 9 and Propidium Iodide (PI), it differentiates the viable ova (stained green with Syto 9) with non-viable (stained red with PI). Karkashan et al. (2015) reported this method could improve the viability assessment of helminth ova up to 85%. There is, however, potential of generating faulty images because of various amounts of DNA, which both dyes bind (after penetration
through intact structures of cells), emitting fluorescence, resulting in difficulty differentiating ova for visual assessment.

The method still requires skilled personnel to differentiate helminth ova with similar morphology. For example, hookworm and Ascaris ova of different species are morphologically identical and cannot be differentiated with microscopic observation (Traub et al. 2004, 2007; Do et al. 2007). Previous studies have noted that even skilled personnel may not be able to distinguish hookworm ova of different species as well as from other helminths (Cabaret et al. 2002; Traub et al. 2007; Verweij et al. 2007). Therefore, results obtained from the vital stain method may not be accurate and reliable in measuring the health risks.

**Molecular methods**

Developments in PCR methods have resulted in rapid, specific and sensitive detection of helminth ova from environmental samples (Gyawali et al. 2015b; Rudko et al. 2017). A high number of gene copies per ovum (Pecson et al. 2006; Raynal et al. 2012; Gyawali et al. 2017a) allows molecular method to detect less than an ovum from wastewater (1 L) and sludge (4 gm) samples. Gyawali et al. (2015b) demonstrated that the real-time PCR method has the ability to detect <1 ova from 1 L of treated wastewater. This means that the acceptable limit of viable helminth ova in treated wastewater and sludge for unrestricted use can be lower to <0.1 in treated wastewater (1 L) and sludge (4 gm) as suggested by many health regulators (IWMI and IWRC 2010). Additionally, ribosomal rRNA or rDNA of Internal Transcribed Spacer (ITS-1 and ITS-2) DNA regions contain high variability in a closely related species (Traub et al. 2007, 2008), thus can be used to distinguish human and zoonotic species from environmental samples.

Despite high sensitivity and specificity, the real-time PCR method is not able to quantify the concentration of helminth ova. A quantitative PCR (qPCR) method, however, can quantify helminth on the basis of amplified gene copy numbers present in the target nucleic acid (Pecson et al. 2006, Verweij et al. 2007, Traub et al. 2008, Ngui et al. 2012, Gyawali et al. 2016a, Gyawali et al. 2017a, Gyawali et al. 2017b).

<table>
<thead>
<tr>
<th>Detection methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture-based</td>
<td>• Viability is possible</td>
<td>• Prolonged time to obtain a result</td>
<td>de Victorica &amp; Galvan (2003), Bowman et al. (2003), US EPA (2003), Gyawali et al. (2017b)</td>
</tr>
<tr>
<td></td>
<td>• Cheaper chemicals and equipment</td>
<td>• Suitable conditions and regular observation necessary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Can be done in a small scale laboratory</td>
<td>• Low sensitivity and specificity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Faster than culture-based method</td>
<td>• Possibility of false positive result because stain may not penetrate the cell wall of recently inactivated ova</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fewer chemicals and equipment</td>
<td>• Differentiation between the species is impossible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Can be done in small scale laboratory</td>
<td>• Due to the toxicity of stains, ova may be inactivated during staining process</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Viability can be assessed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular</td>
<td>• Quick and easy process</td>
<td>• Require advanced laboratory and equipment</td>
<td>Pecson et al. (2006), Verweij et al. (2007), Traub et al. (2008), Ngui et al. (2012), Gyawali et al. (2016a), Gyawali et al. (2017a), Gyawali et al. (2017b)</td>
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<td></td>
<td>• High sensitivity and specificity</td>
<td>• Genomic information is essential</td>
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<tr>
<td></td>
<td>• Automated process</td>
<td>• Possibility of providing false positive result by extracting DNA from inactivated ova</td>
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<tr>
<td></td>
<td>a) Multiple species can be identified from single sample</td>
<td>• Possibility of false negative result via inhibitors present in the samples</td>
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<tr>
<td></td>
<td>b) Quantification is possible</td>
<td>• Sensitivity could be reduced because of multiple sets of primers</td>
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<tr>
<td></td>
<td>c) Viability can be assessed</td>
<td>• Due to the toxicity of stains, ova may be inactivated during staining process</td>
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</tbody>
</table>

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a) – Multiplex PCR.
b) – Quantitative PCR.
c) – PMA-qPCR.
demonstrated that the application of the chemometric statistical analyses is able to characterise and differentiate large groups of biological data based on their metabolic profiles (Kouremenos et al. 2014; Tyagi et al. 2015). This was also demonstrated by analysing an untargeted metabolic profiling of human faecal samples infected with Cryptosporidium spp. (Ng et al. 2012). Gyawali et al. (2016b) also demonstrated the potential application of mass spectrometry and chemometric analysis for distinguishing viable and non-viable hookworm ova in a laboratory setting. There are, however, many challenges to the widespread application of multiomics approach within the context of the areas/countries impacted (third world endemic regions).

Firstly, this approach is relatively expensive with the minimal cost for equipment being 200,000 NZ dollars. Secondly, it requires advance laboratory and well-trained personnel to run the assays and analyse the data. Despite the challenges, this approach has the potential to identify key biological marker that can be used to develop a low cost devices such as strip, aptamers or bio-sensor in future that can be used for the detection of helminths from wastewater in real time.

REFERENCES


FUTURE DIRECTION

Multiomics is the analysis of genome, proteome, lipidome and metabolite information of an organism. It is the most rapidly advancing technology and has the potential to identify key markers (gene, protein, lipid and metabolites) that influence the development and infectivity of a pathogen (Preidis & Hotez 2015; Tyagi et al. 2015). Viability could be determined by identifying low molecular weight intracellular chemical compounds. Furthermore, studies have


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