GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

TRICHURIS TRICHIURA

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Summary

Trichuris trichiura is a metazoan parasite in the group of geohelminths, which means that transmission of eggs is often from contaminated soil. Gravid *Trichuris trichiura* females lay 3,000-20,000 unembryonated eggs every day that are excreted in the host feces. Humans are the only known hosts of *T. trichiura*. Following excretion, fertilised *Trichuris* eggs will mature into an embryonated infective stage, which, if consumed by a susceptible person will establish a new cycle of infection. Worldwide it has been estimated that 600-800 million persons are infected by this nematode. Nevertheless, this may be a conservative estimate as just 10% of infected subjects present symptoms. Level of infestation and susceptibility may also vary based on predisposing genetic factors in the human host. School-age children show higher prevalences, most likely due to increased exposure to contaminated soil and partial natural immune protection after repetitive exposures. Genetic predisposition has been reported in some populations.

Clinical manifestations vary and depend on the degree of infection. Light infections are usually asymptomatic, whereas heavy infections may manifest with watery, mucus-laden, bloody, painful diarrhea, which may progress into anemia and growth retardation if not treated. Rectal prolapse is also associated with heavy infections. Diagnosis is made by microscopic examination of stool samples and identification of unembryonated eggs. Trichuriasis is effectively treated with albendazole and alternative treatments include mebendazole, levamisole, ivermectine and pyrantel.

Warm, damp soil provides the best medium for egg development and transmission, and although there may be some transmission in temperate areas, in tropical areas hyperendemic transmission may occur. Water may be vehicle of transmission when it is contaminated by ova previously embryonated in soil or mud. Consequently, water containing embryonated ova, as well as vegetables irrigated with sewage or sewage effluent, may act as vehicles of transmission.

Preventive measures at the primary level include proper sanitation, safe water, and food safety. Inactivation of embryonated ova by the use of urea and solar heat in bio-waste has been proposed to inactivate ova. Education in hygienic practices is complementary to water and sanitation. In the secondary level of prevention, mass chemotherapy among school age children has been highly successful in its control, but due to heavy environmental contamination may only provide a temporary reprieve before reinfection.

Geohelminths, also known as soil-transmitted helminths (STHs), are metazoan parasites that require soil to complete their life cycle (Guerrant et al., 2011). *Trichuris trichiura* is the second most common geohelminth in the world (Pullan et al., 2014); although this may be changing since *T. trichiura* has become the most prevalent STH, at

least in some regions of the world (Yu et al., 2016; Al-Mekhlafi et al., 2006; Norhayati et al., 1997). Other members in this classification include *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Necator americanus* (Pullan et al., 2014). Because of this geographic and demographic distribution, trichuriasis is classified as a Neglected Tropical Disease (NTD) (Mackey et al., 2014). Neglected Tropical Diseases are a group of conditions that are highly prevalent among impoverished and marginalized tropical populations (Fitzpatrick and Engels, 2016; Mackey et al., 2014) and for which there is insufficient funding to either conduct proper research or to implement appropriate elimination and control strategies (Mackey et al., 2014).

Trichuriasis, the disease caused by the colonization and infestation of the human intestine with whipworms, is widely distributed worldwide with higher prevalence in usually impoverished tropical and subtropical regions (Guerrant et al., 2011). *Trichuris* spp. eggs are excreted in feces, end up in sewage but they need soil or soil-like storage conditions (e.g. like in sludge) to achieve its infective embryonated stage.

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

Trichuris trichiura has a worldwide distribution (Figure 1). Along with *Ascaris lumbricoides* and hookworms, *Trichuris trichiura* is one of the three most prevalent STHs in the world (Cooper et al., 2011). It is estimated that more than two billion people in the world are infected with at least one of these soil-transmitted helminths (WHO, 2016; Savioli et al., 2005; Pullan et al., 2014). Nevertheless, this may be a conservative estimate considering that only 10% or less of infected people present symptoms (Guerrant et al., 2011). In fact, some authors have suggested that the underestimation of *T. trichiura* is related to these four factors: 1) most people harbor low intensity infections which are asymptomatic; 2) it usually is found as one agent within a multi-agent contamination; 3) symptoms onset is particularly slow which may lead to lack of awareness among family members; and, 4) *Trichuris* produces disease in children rather than adults (Guerrant et al., 2011). In addition, the level of infestation and susceptibility may also vary based on predisposing genetic factors in the human host (Williams-Blangero et al., 2008). Early studies had suggested that a genetic component was responsible for some of the predisposition to trichuriasis (i.e. for 28-36% on the egg count variation) as evidenced both by: 1) higher levels of trichuriasis among the population in the Jishan Island (China) as compared to the Jirel population in Nepal; and, 2) by the high household egg count correlation among relatives with lack of household correlation among non-relatives (Williams-Blangero et al., 2002). Subsequently, researchers provided evidence that 2 quantitative trait loci on chromosomes 9 and 18 may be responsible for the susceptibility to *T. trichiura* infection in some genetically predisposed individuals from a Tibeto-Burman ethnic group (i.e. the Jirel population in Nepal) (Williams-Blangero et al., 2008).

Figure 1. Worldwide Prediction of *Trichuris trichiura* Prevalence, 2014. Data source: Global Atlas of Helminth Infections. STH/SCH Web Map App.

Worldwide, *Ascaris lumbricoides* continues to be the most prevalent STH infecting an estimated 683 to 861 million people compared to 427 to 503 million cases of trichuriasis during 2015 (Disease et al., 2016). However, in the Americas, *Trichuris trichiura* has replaced *Ascaris lumbricoides* as the most prevalent STH with 100 million inhabitants infected with the former compared to just 84 million people infected with the latter (Bethony et al., 2006). Similarly, *Trichuris trichiura* prevalence is higher than that of *A. lumbricoides* both in the East and Southern Sub-Saharan Africa (SSA) regions (Pullan et al., 2014). It is estimated that 42.2 million cases of *Trichuris trichiura* occur in the East SSA Region compared to 34.4 million cases of *Ascaris lumbricoides* whereas in the Southern Region *Trichuris trichiura* is the most prevalent STH and accounts for 23.3 million cases versus 8.6 million cases of *Ascaris lumbricoides* (Pullan et al., 2014). In countries like Malaysia, *Trichuris trichiura* is the most common STH representing 57% of all helminthic infections among indigenous tribes followed by *Ascaris lumbricoides* with 24% (Anuar et al., 2014). Although this may be a result of the successful school-based deworming activities in addition to improvements in water and sanitation, it may also be an indication of the development resistance to benzimidazole anthelmintic drugs in *Trichuris trichiura* parasites. Additionally, albendazole-only, ivermectin-deficient therapeutic regimes might not be effective in clearing *Trichuris trichiura* (Hotez, 2009).

1.1.1 Global distribution

Trichuris trichiura is distributed worldwide with areas of hyper-endemicity located in tropical zones. Geographic-information systems (GIS) are increasingly finding application in global public health research. GIS-based analyses have successfully been conducted in a wide range of communicable diseases. This novel tool has the potential to detect and characterize geographic areas with soil characteristics propitious for an intense STH transmission and then predict and identify other unknown areas that may favor parasite growth and development.

1.1.1.1 Groups at risk

People living in tropical and subtropical areas of the world are at highest risk of infection by *T. trichiura*; however, populations without reliable access to safe water and sanitation elsewhere are also at increased risk of infection (Guerrant et al., 2011). The observed increased incidence of this parasite in warm areas of the world may be associated with the capacity of the ova to continue its development in soil under favorable temperatures (usually between 28 and 35°C) until it reaches its infective stage (Moe and Izurieta, 2003). As a matter of fact, it has been previously reported that helminths' ova can stop its development in the environment if they do not find favorable temperature and moisture conditions (Manser et al., 2015).

Trichuriasis is a disease that most commonly affects

school-age children, (Bethony et al., 2006) who show higher prevalence (Yu et al., 2016; Bethony et al., 2006; Corrales et al., 2006) most likely due to increased exposure to contaminated soil and partial natural immune protection after repetitive exposures (Bethony et al., 2006). Since the disease is rarely severe among adults and since it is widely distributed among impoverished populations in the rural tropics, it is considered to be an NTD, as explained earlier. Among children, chronic and/or repetitive trichuriasis may lead to school impairment, cognitive developmental challenges, poorer performance, and height and weight impairment, among others (Williams-Blangero et al., 2008).

1.1.2 Symptomatology

Clinical manifestations of trichuriasis vary and depend on the degree of infection (Bethony et al., 2006). Light infections are usually asymptomatic whereas heavy infections may manifest with watery, mucus-laden, bloody, painful diarrhea, which may progress into anemia and growth retardation if not treated (Bethony et al., 2006; Guerrant et al., 2011). In severe cases, a mucopurulent dysenteric diarrhea may be accompanied by rectal prolapse, as shown in Figure 2 (Jung and Beaver, 1951). There is usually no eosinophilia. In severe pediatric cases, digital clubbing, hypoproteinemia, severe anemia and evident growth retardation are present (Cook and Zumla, 2003).

Figure 2. Rectal prolapse caused for massive *Trichuris trichiura* infestation. Centers for Disease Control and Prevention. Public Health Image Library - PHIL 13370. Atlanta, GA - USA. <https://phil.cdc.gov/Details.aspx?pid=13370>

1.2 Taxonomic Classification of the Agent

Trichuris trichiura is a nematode that belongs to the Trichocephalida order (Myers and Espinosa, 2017) and, therefore, it is related to *Trichinella spiralis*. A more detailed taxonomic classification of this nematode is offered in Table 1. As shown in Table 1, *Trichuris trichiura* is classified within the *Trichuris* genus, a genus that has a complex and controversial species-level classification and which was first reviewed by Dujardin in 1845 (Callejon et al., 2015). Several species have been described, specially among ruminants, many with ability to infect more than one

host (Callejon et al., 2015). By 1974, more than 23 ruminant *Trichuris* species were described by Knight (Callejon et al., 2015). *Trichuris* species differentiation and classification is extremely complex, to say the least, and different approaches have been used including physical differentiation (i.e. based on spicule sheath, spicule length, the spines in the spicule sheath, vulvar structure, presence or absence of vulvar spines, vaginal shape, length and structure), electronic microscopic differences, isoenzymatic analysis, molecular techniques, genetic sequencing, etc. (Callejon et al., 2015; Ketzis, 2015).

Table 1. Taxonomic classification of *Trichuris trichiura*. Data Source: University of Michigan's Museum of Zoology (http://animaldiversity.org/accounts/Trichuris_trichiura/classification)

Taxonomic Level	Classification
Domain	Eukarya
Kingdom	Animalia
Phylum	Nematoda
Class	Adenophorea
Subclass	Enoplia
Order	Trichocephalida
Family	Trichuridae
Genus	<i>Trichuris</i>
Species	<i>trichiura</i>

Many other species of *Trichuris* have been found to infect other mammals, including: *T. campunala* and *T. serrata* in cats (Ketzi, 2015), *T. globulosa* in camels (Callejon et al., 2015), *T. tenuis* in camelids, *T. ovis* and *T. skrjabini* in sheep (Callejon et al., 2015), *Trichuris discolor* in cows, *Trichuris ovis* in sheep and goats, and *Trichuris leporis* in rabbits (Faust, 1949). Consequently, the detection of *Trichuris* spp. ova in soil, sludge, or biosolids can be associated with its contamination with feces from humans or from any of the other mammals that can be infected by a trichurid parasite.

1.2.1 Physical description of the agent

1.2.1.1 *Trichuris trichiura*

Trichuris trichiura exists in two main life cycle stages: eggs and adults, with larvae representing the intermediate phase between eggs and adults.

1.2.1.1.1 Ova

Trichuris trichiura thick-walled, barrel-shaped eggs are yellow to brown in color (Figure 3) and relatively small when compared to other human nematodes (*T. trichiura* eggs are 54 µm in length and 22 µm in width: range 49-65 µm and 20-29 µm, respectively) (CDC, 2016; Guerrant et al., 2011). One important morphologic characteristic of *T. trichiura* eggs is the presence of a polar mucoid "plug" at each extreme, although in rare occasions atypical eggs lacking these plugs may be found (CDC, 2016). *Trichuris trichiura* eggs may depart from their ellipsoidal shape and become thinner or fatter. The embryo within the eggs may be found in any of the following developmental stages: underdeveloped, partially developed, fully developed or death stage (Price, 1994).

Figure 3. *Trichuris trichiura* ova structure. (Courtesy of University of South Florida Donald Price Center)

1.2.1.1.2 Adult parasites

The adult parasite is shaped like a whip, hence its common name: whipworm (Guerrant et al., 2011). The adult parasite is 40 to 45 mm length in the male and 30 to 35 mm length in the female, and shows a greyish-white often slightly pink color. Adult *Trichuris* parasites display a hair-like structure in their heads; however, it was originally described as being located in their tails and therefore the name *Trichuris* where tricho means hair and uris means tail (Guerrant et al., 2011). The finer part of the whip is the anterior end (also called stichosome) whereas the wider part (i.e. the handle of the whip) corresponds to the posterior end (Guerrant et al., 2011). The posterior end hosts the reproductive organs and the intestine whereas the pharynx is found in the anterior part (Guerrant et al., 2011). Among males, the cellular esophagus is half as long as the posterior end, which is has a caudal extremity 360 degrees coiled and hosts the testis, ejaculatory duct, spicule and

cloaca. The female parasite has half of the posterior portion occupied by a stout uterus full of eggs (Price, 1994). In cases when the infection is untreated, the worm can live between 4 to 8 years in the host (Markell et al., 1999).

1.2.1.2 Other trichurid nematodes

Ova of *Trichuris vulpis*, a parasite of dogs that can mature in humans, can be differentiated from that of *Trichuris trichiura* because of its larger size (70 to 80 x 25 to 30 µm) (Figure 4) (Gorbach et al., 1992; Price, 1994). The ova of *Trichuris suis*, the trichurid species that infects pigs, are similar to the ova of *Trichuris trichiura* and they can also infect humans (Cook and Zumla, 2003). As a matter of fact, an increase incidence of this parasite has been observed in semirural households, which practice pig farming (Corrales et al., 2006).

Figure 4. *Trichuris vulpis* ova structure (Courtesy of University of South Florida Donald Price Center)

1.2.2 Tissue tropism/cellular receptors/latency

Trichuris trichiura and other trichurid parasites induce a Th2 immune response. This characteristic has led to the experimental use of *Trichuris suis* ova for the treatment of Crohn's disease in humans (a condition which is characterized by a strong Th1 response) (Summers et al., 2005; Weinstock and Elliott, 2009). The rationale for this approach lies in the fact that Th1 and Th2 immune responses are counterbalancing arms of the immune system (Wright et al., 2009). Although Th2 responses have clearly been associated with *Trichuris trichiura*, recent reports suggest that chronic and/or heavy geohelminth infections may lead to a dampened, regulated immune

response that has been called modified Th2 response (Figueiredo et al., 2010). Modified Th2 responses had previously been described with high-level exposure to allergens, specially cat allergens (Platts-Mills et al., 2001). It seems, that there exist a self-regulating mechanism through which the immune system balances out the harmful inflammatory Th2 responses should an immunogen be present in excess quantity or time. Similarly, recent studies suggest that chronic/heavy infections with geohelminths may lead to a modified Th2-like response (Reina-Ortiz et al., 2011); which, in theory, would have a protective effect. Modified Th2 responses are characterized by a cytokine pattern typical of a Th2 response (i.e. abundant IL-4) with the addition of the immunomodulatory IL-10 cytokine (Sanchez et al., 2015; Platts-Mills et al., 2001).

1.3 Transmission

1.3.1 Life cycle

1.3.1.1 in the human and animal host

Adult female *T. trichiura* dwell in the mucosal crypts of the cecum and lay between 2,000 and 20,000 unembryonated eggs (Urquhart et al., 1988; Guerrant et al., 2011). The number of ova per worm excreted in feces is in inverse relation to the number of worms (Bundy and Cooper, 1990). Soil contamination with geohelminth eggs is an essential step in the chain of infection. In the soil, eggs need to mature or embryonate, a process that takes between 15 and 30 days (i.e. 2-4 weeks) (Guerrant et al., 2011), a timeframe that varies according to environmental conditions (Tun et al., 2015). Soil egg maturation is a sequential process during which *Trichuris trichiura* evolves from unembryonated egg into a 2-cell stage, then into advanced cleavage stage and, finally, into infective embryonated eggs which will continue the life cycle in a

human host if ingested. Larvae in stage L1 are found within embryonated *T. trichiura* eggs (Guerrant et al., 2011). After ingestion, larvae will be released from embryonated eggs, hatching in the small intestine. It has been suggested that this step is at least partially regulated or induced by the presence of intestinal microbiota (Giacomin et al., 2015). Larvae will mature through 4 phases (i.e. up to L4 stage larvae) to develop into large intestine-dwelling adults (both male and female), which will reside in either the cecum and/or the ascending colon (Guerrant et al., 2011). In this habitat, *T. trichiura* inserts its stichosome into the cryptal epithelium whereas the posterior end floats freely in the intestinal lumen (Guerrant et al., 2011). Sixty to seventy days after infection, females start laying eggs and the cycle repeats itself. Adult *Trichuris trichiura* may live for up to 1-3 years (Guerrant et al., 2011). A summary of the life cycle is offered in Figure 5.

Trichuriasis

(*Trichuris trichiura*)

Figure 5. *Trichuris trichiura* life cycle. Source: da Silva and Moser, 2002. This is an illustration of the life cycle of *Trichuris trichiura*, the causal agent of Trichuriasis. Public Health Image Library - PHIL 3424. Centers for Disease Control and Prevention. Atlanta, GA - USA. <https://phil.cdc.gov/Details.aspx?pid=3424>

1.3.1.2 In the environment

As explained earlier, soil egg maturation is a sequential process during which *Trichuris trichiura* evolves from unembryonated egg into embryonated eggs, which will continue the life cycle in a human host, if ingested. Detailed survival conditions and development of eggs in the environment are described in section 2.0 Occurrence in the Environment.

1.3.2 Hosts and reservoirs

Soil is a reservoir for all *Trichuris* species. Humans and non-primate animals such as pigs and dogs are reservoirs for *Trichuris trichiura*, *Trichuris suis* and *Trichuris vulpis*, respectively. *Trichuris* spp. are helminths that infect the ceca and colons of a variety of mammalian species including anthropoids, swine and canine. *Trichuris trichiura* parasitizes human and nonhuman anthropoids (Kuntz and Meyers, 1966; Melfi and Poyser, 2007). In fact, *Trichuris trichiura* is easily transmitted between human and non-human primates including monkeys and lemurs

(Stephenson et al., 2000). More specifically, *T. trichiura* has been reported in *Colobus ruformitratus* and *Cercopitheccus diana* monkeys (Faust, 1949). Therefore, it should be classified as an anthroponosis with important impact in human/public health.

1.3.3 Animal hosts

It has been previously suggested that stray cats and dogs may serve as important sources of environmental contamination for helminth infections (Tun et al., 2015). In fact, a study conducted in Malaysia revealed that 87.7% of stray dogs and 57.9% of stray cats were found to harbor a helminth infection with 8.4% of those infections being caused by *Trichuris* spp. (Tun et al., 2015). This level of infection may hold a public health relevance given the fact that stray cats and dogs tend to defecate within an urban/human environment increasing risk of infection to other healthy animals (i.e. mascots) or even humans (Tun et al., 2015). The same study by Tun et al. (2015) revealed that 23% of soil samples from recreational parks and other frequently visited locations such as bus stops, night

markets, streets and children's playgrounds were infected with helminth eggs.

1.3.4 Pathogenesis and immunity

There are numerous pathogen- and host-derived molecules that play an important role in the pathogenesis of, and subsequent immune responses to, *Trichuris trichiura* infections. Serine-proteases are a family of ubiquitous multifunctional, multiorganism proteins that are believed to be involved in the pathogenesis of parasitic helminths, among other functions, contributing as crucial factors for the successful colonization/establishment of an infection (Yang et al., 2015). Specifically, *Trichuris muris*, the mice trichurid parasite, expresses two major collagen-specific serine peptidases (i.e. serine peptidases with activity against collagen-like molecules) which are believed to be involved in the host tissue invasion process including the development and maintenance of the parasite's characteristic muco-syncytial habitats as well as the penetration or integrity disruption of epithelial cell membranes (Yang et al., 2015). Moreover, it has been suggested that *T. muris*-derived serine proteases can cleave Muc2, a major intestinal mucin, leading to mucin network disassembly and, therefore, to impaired mucus layer production which may translate in lower probability of parasite expulsion (Yang et al., 2015). Another putative function of this serine-protease would be the creation of an environment conducive to mating and egg laying (Yang et al., 2015).

Upon helminth infection, host immune responses are triggered to get rid off the infection. Typically, acute helminth infections are followed by strong, inflammatory Th2 responses that are characterized by an IL-4 lead immune environment. In fact, *Trichuris trichiura* infections have been associated with Th2 immune responses (Giacomin et al., 2015). However, it has been hypothesized that chronic infections might lead to a dampened version of this Th2 response that would aim at targeting the parasite while preventing the harmful effects that chronic inflammation may have on the hosts tissues. Such response has been labeled as modified Th2 response and is characterized by increased IL-10 levels in the context of the high IL-4 Th2 response. Modified Th2 responses have been observed in other circumstances of chronic exposure to Th2-inducing antigens, like cat-derived allergens (Platts-Mills et al., 2001). Similarly, recent studies have suggested that such Modified Th2 response might be evoked not only during chronic but also in heavy infections, which may also elicit a harmful, strong Th2 response (Reina-Ortiz et al., 2011).

Interestingly, Th2 responses are believed to downregulate Th1 and Th17 immune responses, which in turn are associated with inflammatory bowel disease (Giacomin et al., 2015). Helminth-related inflammation suppression is not an exclusive characteristic of *Trichuris trichiura* but rather a more widely observed phenomenon. Although the mechanisms leading to this suppression are not well understood and may include the induction of modified Th2 responses as discussed above,

other pathways have been suggested including a putative helminth-induced microbiota modification (Giacomin et al., 2015). These suppressive characteristics have been argued to be an important factor to consider the potential therapeutic benefits of helminths, specially to treat human intestinal inflammatory diseases such as inflammatory bowel disease or coeliac disease (Giacomin et al., 2015). Experimental data supports this hypothesis. For instance, *Heligmosomoides polygyrus bakeri* infection lead to increased lactobacilli and improved outcomes among IBD suffering mice whereas TSO administration lead to reduced *Fibrobacter* and *Rumicoccus* with a concomitant increase in *Campylobacter* in pigs (Giacomin et al., 2015). Similarly, *Trichuris trichiura* administration to idiopathic chronic diarrhea (ICD)-diseased macaques lead to reduced cyanobacteria with increased bacterial diversity including rise in *Bacteroidetes* and *Tenericutes* (Giacomin et al., 2015). Interestingly, *Trichuris* treatment affected bacterial attachment which suggests that whipworm infection might have a role in mucosal healing (Giacomin et al., 2015).

Other mechanisms of immune regulation associated with whipworm infection include following: Interferon gamma and interleukin (IL) 17 suppression, increased type 2 cytokine production, regulatory T cell and immunomodulatory cytokine (i.e. IL-10, IL-22, Transforming Growth Factor - beta) induction, and recruitment of alternatively activated macrophages, dendritic cells and B cells (Giacomin et al., 2015).

1.3.5 Other trichurid nematodes

1.3.5.1 *Trichuris suis*

Trichuris suis is the porcine whipworm. Pigs and wild boars are the natural host for *Trichuris suis*, a parasite that can also affect other species - including transient, asymptomatic infections in humans (Nejsum et al., 2012; Giacomin et al., 2015). Humans probably become infected with *T. suis* by ingesting contaminated soil or water. Due to this double benefit (i.e. transient asymptomatic infections leading to inflammation suppressive immune responses), *Trichuris suis* ova (TSO) have been tried as a therapeutic solution for Crohn's disease, ulcerative colitis and multiple sclerosis (Giacomin et al., 2015). *T. suis* is found worldwide, but is most prevalent in warm, humid climate whereas it is rare or nonexistent in arid, very hot, or very cold regions (The Center for Food Security and Public Health, 2005). *T. suis* adults survive in pigs for approximately 4 to 5 months.

1.3.5.2 *Trichuris vulpis*

Trichuris vulpis are the canine trichurid species. Although *T. vulpis* may be found worldwide, these parasites are most prevalent in warm and humid zones, similar to observed distribution of *Trichuris trichiura*. Similar to *T. suis*, *T. vulpis* are rarely found or are nonexistent in arid, very hot, or very cold regions. Human cases of *T. vulpis* have already been reported as early as 1956 (Hall and Sonnenberg, 1956) and this parasite should also be considered a zoonotic disease for man (Kagei et al., 1986).

T. vulpis-associated diarrhea has been reported among slum-dwelling children of India (Mirdha et al., 1988), an occurrence that might be common in other overcrowded urban locations, (Stephenson et al., 2000) whereas epistaxis and abdominal discomfort were reported in a 9 years old girl from Mexico (Marquez-Navarro et al., 2012). Additional human cases have been suspected or reported in Italy (De Carneri et al., 1971), Poland (Lineburg and Jastrzebski, 1987) and the United States (Dunn et al., 2002). *T. vulpis* adults survive in dogs for approximately 16 months (The Center for Food Security and Public Health, 2005). A prevalence of up to 10% has been reported in both stray and cared-for dog populations in various countries (Robinson et al., 1989; Vanparijs et al., 1991). However, *T. vulpis* has not been a frequent laboratory report in human hosts, which may indicate a possible misclassification with *Trichuris trichiura* during coprology diagnosis.

1.4 Population and Individual Control Measures

1.4.1 Vaccines

There are two factors to consider in the development of a *T. trichiura* vaccine. First, the pick of prevalence of *T. trichiura* in school-age children (Yu et al., 2016; Bethony et al., 2006; Corrales et al., 2006) makes it plausible to propose the development of natural immune protection of children after various exposures to this parasite. Second, anthelmintic treatment of school-age populations is one of the main, if not the main, public health strategy implemented in the control of *Trichuris trichiura* and other helminths (Kepha et al., 2017). This therapeutic control of *T. trichiura* may prevent the development of active natural immunity and may lead to drug resistance. Consequently, the development of a *T. trichiura* vaccine, in addition to water and sanitation, can represent more sustainable interventions in the control of this parasite.

Various research teams are currently working in the identification of functional antigens against *Trichuris muris*. These preliminary studies may be the initial steps for the development of a *Trichuris trichiura* vaccine for humans (Dixon et al., 2008; Dixon et al., 2010). The most effective antigenic structures are the anterior (esophageal) region of the adult worm, although protection was apparent following vaccination with antigens prepared from other regions of adult worms and from whole larval worms (Wakelin and Selby, 1973; Dixon et al., 2008). Most recently, the development of a pan-anthelmintic vaccine against *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms is on experimental phases at The Sabin Vaccine Institute Product Development Partnership (Zhan et al., 2014). Nevertheless, urticarial reactions upon receiving recombinant forms of such antigens have been reported (Diemert et al., 2012). These issues must be addressed since, at least for some antigens, it has been previously reported that allergic reactions may lead to life threatening anaphylactic reactions upon antigen re-exposure in susceptible individuals (Stratton et al., 1994).

1.4.2 Hygiene measures

There are several easy-to-implement, common hygiene practices that can be adopted to prevent *Trichuris* spp. transmission. In fact, access to water and hygiene sanitation have been identified as factors affecting STH transmission intensity (Kepha et al., 2017). Proper wash of hands, vegetables and fruits can reduce the risk of acquiring helminth infections. However, this practice may be insufficient if vegetables or fruits that are being consumed raw have already been contaminated by soil or wastewater in the environment. The double and strong chitin structure of helminth ova may render the application of chlorine useless (i.e. chlorine may not be effective for helminth ova inactivation) (Guadagnini et al., 2013).

1.4.3 Diagnosis and treatment in humans

Trichuris trichiura diagnosis in humans is made by microscopic examination of stool samples and identification of unembryonated eggs. Although *T. trichiura* eggs may be easily identified in a microscopic preparation given their particular morphologic characteristics (i.e. the usual presence of a “plug” at each end), sometimes eggs might be vertical or inclined in their orientation resulting in a more difficult observation. This inconvenience may easily be corrected by gently tapping the coverslip (CDC, 2016).

There are several techniques used for stool sample examination. The most widely used in clinical practice probably is fresh examination whereas the most useful for epidemiological surveys, based on their increased sensitivity, would include Kato-Katz and filtration/concentration techniques (Guerrant et al., 2011).

Treatment of *Trichuris trichiura* in humans have traditionally been accomplished through the use of benzimidazole derivatives. In fact, very often, Trichuriasis is effectively treated by 400 mg of albendazole given orally as a single dose. Alternative treatments include mebendazole, levamisole, ivermectin and pyrantel. However, Mebendazole and Albendazole inefficacy for the treatment of infections with *Trichuris trichiura* has been confirmed in various studies (Levecke et al., 2014b; Bennett and Guyatt, 2000; Keiser and Utzinger, 2008; Speare et al., 2006). This resistance has led to the evaluation of new drugs or drug combinations against *Trichuris trichiura* including pyrantel/oxantel (Albonico et al., 2002), mebendazole/ivermectin (Knopp et al., 2010), oxantel, (Speich et al., 2014), and papaya cysteine proteinases (Levecke et al., 2014a).

1.4.4 Mass drug therapy

According to recent studies, it is expected that deworming campaigns with a single oral dose of mebendazole (500 mg) among school-age children could lead to a greater than 50% fecal egg count reduction (FECR) rate. These estimates are based on the results of six trials involving 5,830 school children in Brazil, Cambodia, Cameroon, Ethiopia, the United Republic of Tanzania, and Vietnam. Should FECR rates be lower than this level,

potential development of drug resistance may be suspected (Levecke et al., 2014b).

1.4.5 Management of contacts

Preventive measures at the primary level include proper sanitation, safe water, and food safety. Inactivation of embryonated ova by the use of urea and solar heat in bio-waste has been proposed to inactivate geohelminths ova (Sharad et al., 2012). Education in hygienic practices such as hands washing, thorough washing of vegetables, and proper cooking of foods potentially contaminated with embryonated ova are also important. There is no vaccine available until now (please refer to vaccine section). In the secondary level of prevention, mass chemotherapy among school-age children has been highly successful in its control.

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods in the Environment

The detection of ova in the environment remains a challenge due to the diluted concentrations with which they are found in sludge, biosolids or wastewater. Novel methods for detection have been developed based on biomolecular diagnosis like PCR. Nevertheless, these methods may not be able to differentiate between viable and non-viable ova. The US Environmental Protection Agency (EPA) has developed their own standard method based on a process of sedimentation, flotation, isolation and incubation of ova (EPA, 1999). Additional similar techniques have been developed, including Membrane filter, Leeds I and Faust (Maya et al., 2006). A recent study comparing the US EPA, the Membrane Filter, Leeds I and Faust techniques concluded that US EPA was the most suitable to detect ova in drinking water and wastewater in Mexico (Maya et al., 2006). After filtration, specific identification of helminthes including *Trichina* spp. is done by standard microscopy techniques. Molecular techniques applied to environmental samples, collectively known as environmental DNA or eDNA, (Bass et al., 2015) have also been used to identify ova in sludge, including real-time polymerase chain reaction (RT-PCR), PCR, quantitative real-time PCR (RT-qPCR), nested PCR and loop-mediated isothermal amplification (LAMP) (Amoah et al., 2017; Bass et al., 2015). One of the main challenges for DNA techniques is the difficulty to extract the *T. trichiura* DNA from the hard shell of the ova. Kaiser et al. have proposed the use of ethanol for sample preservation and bead-beating procedure to extract the DNA from the ova shell (Kaiser et al., 2017). The primers most commonly used for PCR techniques to detect whipworms from dogs, pigs, non-humans primates and humans are: Tt_283F, Tt_358R' and Tt_308T_YY (Liu et al., 2013). Unfortunately, DNA techniques do not substantially improve the sensitivity of the traditional microscopic techniques mainly because of the in the preservation of samples and DNA extraction. More recently, innovative techniques have been proposed for ova detection including the use of a microfluidic chambers to isolate ova (Izurieta and Selvaganapathy,

2016) as well as the use of an analytical digital image system to identify ova from wastewater samples processed using the US EPA technique (Jimenez et al., 2016). This analytical digital image system is reported to have a 99% specificity with a 80-90% sensitivity (Jimenez et al., 2016). Finally, the use of low cytometry or droplet digital PCR (ddPCR) for the analysis, detection and/or identification of ova in environmental samples has been proposed although yet to be reported (Amoah et al., 2017).

2.2 Data on Occurrence in the Environment

Trichuris spp. eggs are unembryonated and not infectious when they are excreted. Therefore, direct person-to-person or animal-to-person transmission is not possible. Thus, trichurid eggs must develop in soil, biosolids, or sludge to become infective. Larval development depends on temperature, moisture, presence of solar light, and pH conditions in the soil, sludge or biosolids. If the temperature conditions are optimum (i.e. $28^{\circ}\text{C} \pm 4^{\circ}\text{C}$), first-stage larvae can be developed in 15 to 21 days. If temperature remains constant at 22°C , larva development can take 54 days whereas development can take up to 7 months if the temperature fluctuates between 6°C and 22°C . In the soil, *T. trichiura*, *T. vulpis* and *T. suis* eggs usually remain viable for one year. However, in certain circumstances the eggs can remain viable for years. Humans probably become infected with the anthroponozoonotic *Trichuris* spp. (i.e. swine or canine trichurid parasites) by ingesting contaminated soil or water (Faust, 1949; Bundy and Cooper, 1990).

2.2.1 Raw sewage and sludge

As explained earlier, *Trichuris* spp. eggs are excreted in feces and they need soil or soil-like storage conditions to achieve its infective embryonated stage. Afterwards, soil-embryonated ova may access the human gastrointestinal track through ingestion of contaminated food or water. Consumption of raw vegetables that were irrigated by contaminated water has been described as one of the main mechanisms of transmission. In fact, it has been reported that sewage-contaminated water containing human and animal feces used to irrigate vegetables –estimated to be at least 20 million hectares in 50 countries- cause high prevalence of helminth infections in countries deficient wastewater treatment before it is discharged into rivers, ponds, and lakes (Duedu et al., 2014; Klapec and Borecka, 2012; Kozan et al., 2005).

For instance, a field study evaluating the presence of helminth ova in vegetable samples and which was carried out in Mazandaran province, northern Iran, reported any helminth species 14.95% of samples whereas *Trichuris* spp. ova were found in 2.2% of samples. The amount of *Trichuris* spp. ova found per 200 g of vegetable was 14 in parsley, 13 in lettuce, 9 in spinach, 8 in mint, 7 in radish, and 4 in green onion. The most likely source of contamination in this study was believed to be sewage effluent contaminating river water that is used for vegetable irrigation (Rostami et al., 2016).

2.2.2 Surface water

Although it is possible that unsafe drinking water contaminated with soil or feces may carry *Trichuris trichiura* eggs, these ova are not normally found in piped drinking water systems that are properly maintained. The finding of *T. trichiura* eggs in drinking water is an eventual finding since they are usually removed during the process of decantation and filtration. Nevertheless, eggs may survive in decanted sediments of rivers ponds and lakes (WHO, 2004).

2.2.3 Ground waters and drinking waters

Protected aquifers usually provide microbiological safe water. The probability of ground water contamination by *T. trichiura* eggs is meaningless considering that helminths eggs will be retained during the filtration process. Nevertheless, unprotected and shallow aquifers can be subject to contamination from wastewater discharges and feces containing *T. trichiura* eggs (WHO, 2004).

2.2.4 Seawater and shellfish

Most wastewater that would possibly contain *T. trichiura* eggs is discharged into the ocean, oftentimes close to cities and recreational areas. In fact, a recent study reported that more than 90% of sewage water (approximately 550 megalitres a day in Cape Town, South Africa), which is likely to carry helminth eggs, reaches rivers and, afterwards, the ocean (Adams et al., 2005). The same study reported that helminth eggs might remain viable under the salinity conditions of seawater, (Adams et al., 2005) a hypothesis that has been otherwise proposed and which may have particular importance in island nations like Tuvalu (Speare et al., 2006).

2.3 Persistence

As observed with other geohelminths, warm, damp soil provides the best medium for transmission once it is contaminated with helminth-laden feces. Models of environmental data show that, after controlling for sanitation infrastructure and socioeconomic conditions, humidity, temperature, and pH are associated with *T. trichiura* transmission. Altogether these models suggest that although there may be transmission in temperate areas, intense transmission occurs mostly in tropical areas. Most likely, this observed hyperendemic transmission in tropical areas is associated with favorable conditions for ova development in soil. Only after ova has been embryonated in soil or soil-like surfaces, water has a role as a vehicle of transmission. Consequently, non-potable water containing embryonated ova as well as vegetables irrigated with untreated sewage water are main pathways of transmission in developing countries. As mentioned earlier, *Trichuris trichiura* has been reported in humans and non-human primates and therefore both human and non-human primates act as animal reservoirs whereas other zoonotic trichurid parasites can occasionally infect humans. The role of soil as reservoir has previously been suggested and

described. Soils with warm temperatures (i.e. between 28°C and 35°C) are ideal locations for egg embryonation. On the contrary, temperatures over 35°C (Moe and Izurieta, 2003) and below -80°C (Despommier et al., 1995) destroy eggs.

Infective first stage larvae develop within the egg/shell in 3 to 8 weeks, depending on environmental temperature. The infective egg-encased first stage larvae are highly resistant and can remain in this form for several years in favorable conditions.

3.0 Reductions by Sanitation Management

3.1 Wastewater Treatment

Wastewater has been widely reused for agricultural purposes for a long period of time, especially in developing nations where the need for irrigation surpasses water supply leading to reuse of low quality (i.e. inappropriately treated) wastewater (Maya et al., 2010; Jimenez et al., 2016). Ova concentration in wastewater has been reported to range from 1 (in the United States) to 840 (in Morocco) per liter whereas helminth ova concentration has been reported to range from <1 (in Germany) to 735 (in Egypt) per gram of total solids in sludge (Table 2) (Jimenez, 2007). Helminth eggs present in wastewater can reach soil or crops where the temperature and humidity conditions might be ideal for their development into infective stages (Maya et al., 2010). In wastewater, helminth eggs are the human pathogens most resistant to inactivation during sludge and wastewater treatment (Maya et al., 2012). In addition, helminth eggs are characterized for their high infectiousness (usually one egg can establish infection) and their resistance to commonly used disinfection and/or inactivation techniques (Jimenez et al., 2016). In fact, helminth ova have been characterized as the greatest health risk in wastewater reused for agriculture and aquaculture (Maya et al., 2006; Jimenez, 2007). Accordingly, two out of three main helminth transmission mechanisms are related to wastewater effluent quality (namely ingestion of contaminated crops and direct contact with contaminate wastewater) (Jimenez, 2007). Once they reach crops, helminth eggs can survive for several months, even years; similar to the survivorship observed in water or soil (Jimenez, 2007). This represents both an urgent need and an opportunity we should not miss, especially if we consider that at least 10% of the irrigated land in developing nations, which roughly translates into 20 million ha, is estimated to be irrigated with low quality water (Jimenez et al., 2016). As per World Health Organization (WHO) guidelines, helminth eggs should be found at a concentration of 1 egg / L or less for wastewater irrigation reuse (Jimenez et al., 2016; Maya et al., 2006, 2012). Therefore, the use of proper wastewater treatment techniques is required to ensure that high quality wastewater (effluents) is available for reuse in agriculture and aquaculture. Given the high temperature/low humidity requirements and the estimated relative long time required for inactivation, the goal in wastewater treatment should be helminth ova removal rather than inactivation (Jimenez, 2007).

Table 2. HO concentration in wastewater and sludge. Source: Slightly modified from (Jimenez, 2007)

Country/Region	Wastewater (Ova per Liter)	Sludge (Ova per gTS)
Mexico	6 to 330	73 to 177
Brazil	166 to 202	75
Egypt	ND ^a	Mean: 67; Max: 735
Ghana	ND	76
Morocco	840	ND
Jordan	300	ND
Ukraine	60	ND
United States	1 to 8	2 to 13
France	9	5 to 7
Germany	ND	<1
Great Britain	ND	<6

^aND: Non-detect

Appropriate wastewater treatment may significantly reduce the presence of trichurid parasites. A recent report evaluated *Trichuris trichiura* ova inactivation under different physicochemical conditions using spiked sludge samples. In that study it was observed that *Trichuris trichiura* ova inactivation was directly correlated with increases in temperature, humidity, and pH. For instance, *Trichuris trichiura* ova inactivation at 95%, 90% and 80% humidity is shown in Figure 7, Figure 8 and Figure 9, respectively (Adapted from Maya et al., 2010). This study also demonstrated that, under laboratory conditions, *Trichuris trichiura* eggs are more vulnerable than those of *Ascaris* (Maya et al., 2010).

3.1.1 Composting of fecal wastes

Trichuris spp. ova can be inactivated by manipulating specific storage conditions. Temperatures above 35°C and below -80°C can inactivate the eggs and impede their maturation into infective embryonated forms (Moe and Izurieta, 2003; Despommier et al., 1995). Based on results from field experimental studies done with *Ascaris*

lumbricoides ova, it can be inferred that desiccation or alcalinization do not have the capacity to inactivate *Trichuris* spp. ova in biosolids or in the environment (Moe and Izurieta, 2003; Mehl et al., 2011; Corrales et al., 2006).

3.1.2 Wastewater treatment facilities

Wastewater treatment is a complex process by which effluents are produced with different levels of quality. Primary wastewater treatment consists on the removal of coarse (settleable) solids and large materials through sedimentation, usually with a 25-50% reduction in biochemical oxygen demand (BOD), 50-70% reduction in total suspended solids (TSS), and a 65% reduction in oil and grease (Pescod, 1992). Secondary treatment is applied to primary treatment effluents to remove residual organics and suspended solids (Pescod, 1992). Tertiary treatment, also known as advanced treatment, is used to remove specific components not removed by the previous treatment methods (Pescod, 1992). Table 3 summarizes the main wastewater treatment methods and their important helminth ova removal characteristics.

Table 3. Wastewater treatment methods for generic helminth ova removal. Source: (Jimenez, 2007)

Method	HO Removal Rate	Retention Time	HO Effluent Concentration (HO / L) ^a	Comments / Observations
Wastewater Stabilization Ponds (SP)	>2 log ₁₀	5 to 20 days	NR ^b	Most suitable for agriculture Preferred method in developing nations
Reservoirs & Dams	>2 log ₁₀ ^c	> 20 days	NR	Useful when wastewater is constant but crop water demand is variable
Wetlands	>2 log ₁₀	4 days?	NR	Add horizontal flow gravel bed (25 m length)

Method	HO Removal Rate	Retention Time	HO Effluent Concentration (HO / L) ^a	Comments / Observations
UASB ^d	>2 log ₁₀	5.5 hour	16	Recommended as a complement to SP
CEPT ^e	NR	4 to 6 hours	< 1 to 3	Effluent improves soil productivity
APT ^f	1 to 2 log ₁₀	0.5 to 1 hour	< 1 to 3	Effluent improves soil productivity
Rapid filtration	1 to 2 log ₁₀	7 to 10 m ³ /m ² /h filtration rate	< 1.0	Filter: 0.8 to 1.2 mm, 1 m depth

^aVaries depending on initial concentration; ^bNR: Not Reported; ^cIf operated as batch systems; ^dUASB: Upflow anaerobic sludge blanket; ^eCEPT: Chemical Enhanced Primary Treatment; ^fAPT: Advanced Primary Treatment

Pathogen (i.e. viruses, bacteria, protozoan and metazoan) removal from septic tanks can be primarily achieved through sedimentation (Pescod, 1992). Specifically, helminth ova may be more easily removed because of their weight and size (i.e. a high percentage of the ova will settle). The settling velocity of *Trichuris* spp. ova has been estimated to be 1.53 meters per hour, which is faster than that of *Ascaris* spp. at 0.65 meters per hour but slower than that of *Taenia* spp., which stands at 2 meters per hour (Amoah et al., 2017). However, the small fraction of unsettled helminth ova can survive adverse environmental conditions (e.g. temperature, pH, chemical substances), which would otherwise inactivate other septic tank pathogens like viruses and bacteria (Jimenez, 2007).

There are several secondary and tertiary wastewater treatment procedures that can be applied to remove helminth ova/eggs from primary treatment effluents and, thus, improve compliance with national and international wastewater quality standards. Pescod (1992) classifies these treatments according to their performance into poor, fair or good helminth ova remover. Package Plant, Extended Aeration Activated Sludge and Biological Filter techniques have poor helminth ova removing performance whereas Activated Sludge Plant, Oxidation Ditch and Aerated Lagoon methods are fair removers and Waste Stabilization Pond Systems deemed to be good (highest level) helminth ova removers (Pescod, 1992).

3.1.3 Wastewater secondary/natural treatment

3.1.3.1 Wastewater stabilization ponds and reservoirs

Wastewater stabilization ponds (WSP) have been characterized both as the most suitable wastewater treatment technique for effluent reuse in agriculture and as the preferred wastewater treatment method in developing countries where land cost is reasonable and skill labor lacking (Pescod, 1992). Wastewater stabilization ponds significantly improve removal of bacteria, viruses and protozoa/helminth as compared to conventional methods (Jimenez, 2007). Specifically, stabilization ponds are reported to remove up to >2 log₁₀ of helminth ova compared to 1 to 2 log₁₀ removed using conventional processes

(Jimenez, 2007). Among the several factors leading to this improved removal rate, sedimentation seems to play the most important role (Jimenez, 2007). Retention time to remove helminth ova varies from 5 to 20 days depending on the initial concentration, with majority of ova being retained in the first anaerobic pond (Jimenez, 2007). Similarly, reservoirs and dams are reported to remove helminth ova after >20 days retention time (Jimenez, 2007). Egg removal percentage in WSPs can be calculated by sequentially applying the equation below to each pond in the system:

Equation 1. Egg removal percentage per each sequential pond in Waste Stabilization Pond treatment facilities. Source: (Jimenez, 2007)

$R = 100 (1 - 0.41 e^{-0.49\Theta} + 0.0085 \Theta (exp2))$, where Θ is the retention time

3.1.3.2 Wetlands

Wetlands are a type of Macrophyte Treatment System where aerobic and anaerobic treatment takes place and where BOD and nitrogen/phosphorus/heavy metals are removed by bacterial activity (Pescod, 1992, Jimenez, 2007). Wetlands are reported to remove 0.4 to >2 log₁₀ of protozoans, especially when using a 4-day retention time, whereas >2 log₁₀ helminth ova removal can be achieved by adding a horizontal flow gravel bed in a 25 m length (Jimenez, 2007).

3.1.3.3 Upflow anaerobic sludge blanket (UASB)

Upflow anaerobic sludge blanket (UASB) has been recommended as a complimentary technique to stabilization ponds (Jimenez, 2007). The UASB method uses sedimentation and sludge bed filtration to remove helminth ova (Jimenez, 2007). It has been reported that UASB used with a 5.5-hour retention time produced an effluent containing a mean of 16 helminth ova /L (1.3-45 helminth ova /L) when wastewater containing 64-320 helminth ova /L was used, which represents a 1.3 log₁₀ removal efficiency (Jimenez, 2007).

3.1.4 Wastewater tertiary treatment

In order to raise the quality of the effluent, a wastewater tertiary treatment can be applied. This tertiary wastewater treatment can be accomplished by a variety of methods such as coagulation-sedimentation, filtration, reverse osmosis, and secondary biological treatment to further stabilize oxygen-demanding substances or remove nutrients. In various combinations, these processes can attain any desired degree of pollution control. As the effluent wastewater is purified to higher levels of quality, it can then be reused for: 1) urban, landscape, and/or agricultural irrigation; 2) industrial cooling and processing; 3) recreational uses and water recharge; and, even, 4) indirect and direct augmentation of drinking water supplies (Drechsel, 2010).

3.1.4.1 Coagulation and flocculation

Coagulation and flocculation are important processes derived from ancient techniques and used for drinking water and wastewater treatment (Bratby, 2016). Chemicals can be added to this technique in order to increase the settling velocity of helminth ova (Jimenez, 2007). Chemical Enhanced Primary Treatment (CEPT) refers to the addition of low coagulant doses and high molecular weight / high density charge flocculants to the coagulation-flocculation processes whereas Advanced Primary Treatment (APT) refers to the use of a high rate settler as opposed to a conventional one when conducting CEPT (Jimenez, 2007). Both APT and CEPT achieve an effluent with low concentration of suspended solids and helminth ova while retaining organic matter, nitrogen and phosphorus in the dissolved fraction, (Jimenez, 2007) and probably some other pathogens. This translates into an effluent that improves soil productivity but which still requires disinfection (either UV light or chlorine) to inactivate bacteria (Jimenez, 2007). It has been estimated that effluents with 20-40 mg TSS/L contain around 3-10 helminth ova / L whereas effluents with < 20, mg TSS / L contain less than 1 helminth ova / L (Jimenez, 2007). Retention times vary from 0.5-1 hour for APT to 4-6 hours for CEPT (Jimenez, 2007). A 1 to 2 log₁₀ helminth ova removal can be achieved with APT (Jimenez, 2007).

3.1.4.2 Filtration

Filtration can successfully remove *Trichuris* spp. from effluents of primary or secondary treatment (Landa et al., 1997). For instance, a recent study comparing three macrofiltration methods (namely, Disc Filter, Mesh Filter and Pressure Sand Filter) found that all of them efficiently removed all pathogenic nematode eggs from secondary clarification water samples (Gómez et al., 2010). Pressure

Sand Filter was not only the most efficient in terms of improved turbidity and concentration of suspended solids ($p < 0.0001$) but also the least expensive technique. For non-pathogenic nematode eggs, however, there was only an important reduction in concentration (as opposed to the complete absence observed for pathogenic nematodes), suggesting that there could still be a possibility to recover human pathogenic nematodes after macrofiltration (Gómez et al., 2010). Further research is required to completely rule out the presence of pathogenic nematodes in instances of secondary clarification followed by macrofiltration (Gómez et al., 2010). Nonetheless, these results are encouraging, especially if we consider that nematode eggs are resistant to UV irradiation and disinfection and therefore another step would be required before UV irradiation/disinfection are applied to wastewater if we are to prevent the presence of viable nematode eggs in effluent waters (Gómez et al., 2010). Macrofiltration methods might very well fit this description, as shown by Gómez, et al. (2010). In addition, rapid filtration (> 2m /h) has been shown to remove 1 to 2 log₁₀ of helminth ova, a removal that can be increased by 2 to 4 log₁₀ with the addition of coagulants (Jimenez, 2007). Rapid sand filtration with a specific size of 0.8-1.2 mm, a minimal filter depth of 1 m and filtration rates of 7-10 m³/m²/h yield effluents with constant helminth ova concentration of < 1.0/L (Jimenez, 2007).

3.1.4.3 Reverse osmosis

Recently, a novel pig manure treatment method termed AMAK and which included pressure filtration as its final step reported *Trichuris* spp.-free effluents (Makara and Kowalski, 2015).

3.2 Disinfection

3.2.1 Chemical disinfection with lime, urea, chlorine, and ozone

Lime (CaO), a pH increasing agent, has been used in sludge pathogen inactivation (Maya et al., 2010; Mignotte-Cadiegues et al., 2001). A recent study reported that total inactivation of *Trichuris trichiura* ova can be achieved at different rates depending on specific storage conditions; namely, CaO concentration and humidity (Table 4). In general terms, increasing CaO concentrations and/or decreasing humidity are associated with reduction in the time to achieve total *Trichuris trichiura* ova inactivation.

In other studies, ammonia and CaO have been applied in sludge of open and closed systems. Ammonia was able to remove up to 1.4 log₁₀ of viable helminth ova (Mendez et al., 2002).

Table 4. Time to *T. trichiura* ova inactivation under different CaO concentration and humidity conditions. Source: Created based on data reported by (Maya et al., 2010)

CaO Concentration	Humidity	Storage Time to Total Inactivation of <i>T. trichiura</i> Ova
15%	90%	After 6 months
15%	80%	4 months
20%	90%	After 4 months
20%	80%	2 months

3.2.2 Irradiation and UV disinfection

Trichuris spp. eggs are destroyed by dehydration and sunlight. *T. trichiura* eggs die above 52°C or below -9°C. In the laboratory, *T. muris* eggs can be inactivated with 30% (v/v) ammonia, combined with temperatures greater than 30°C (The Center for Food Security and Public Health,

2005). A recent study reported that inactivation of six helminth species, including *Trichuris trichiura*, can be achieved within 6 days at 45°C, 5.3 pH and 90% dryness (Maya et al., 2012). Helminth egg/ova inactivation is a function of these conditions (i.e. temperature, pH, dryness and time). Table 5 shows the conditions required to inactivate >2 log₁₀ of *T. trichiura* in laboratory experiments. Among all studied helminths, *Trichuris trichiura* and *H. nana* were the least resistant parasites (Maya et al., 2012).

Table 5. *Trichuris trichiura* inactivation under varying conditions in the laboratory^a

Dryness	Non-larval		Larval	
	Contact Time (min)	Temperature (°C)	Contact Time (min)	Temperature (°C)
5%	120	78-80	30	70
5%	180	70-73	60	60
10%	120	70-73	30	60
10%	180	70	--	--
20%	120	66-70	30	60
20%	180	63	--	--

^a Data derived from (Maya et al., 2012)

3.2.3 Biological control by predators

Predatory fungi have been evaluated for it

nematophagous activity against *Trichuris trichiura* eggs. *Pochonia chlamydosporia* isolates (VC1 and VC4) were found to have a role in destroying *Trichuris trichiura* eggs after the 6 hours of observation (Silva et al., 2010, 2011) (Figure 10). Consequently, these fungi could be used as possible biological controls of *Trichuris trichiura*.

Figure 10. Destruction of *Trichuris trichiura* eggs (white arrowx) after the 6-h observation period by isolates of *Pochonia chlamydosporia* (VC1 and VC4) (black arrow). SEM. Bar: (a) 10 µm; (b) 10 µm.

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