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A review on the health outcomes of chronic nematode or Schistosome infections

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Abstract**:** While Schistosome or nematode infections rarely result in major morbidity or death, chronic infections often have insidious consequences. A long-term infection of the major soil transmitted helminths (roundworm, whipworm, and hookworm) can seriously impede a child's growth and cognitive development. Schistosome infections can last up to 10 years, resulting in infertility, cancer, and increased risk of HIV transmission. Filarial infections impair the lymphatic system and can cause lymphedema or elephantiasis. These infections disproportionally burden areas of extreme poverty because they tend to lack adequate access to clean water and sanitation. Interventional strategies, such as mass drug administration, are important measures to continue, but do little to prevent re-infection.

Introduction:

The likelihood of mortality following a helminth or Schistosoma infection is small; of the approximately 2 billion people affected worldwide, the number of annual deaths is likely to be in the hundreds of thousands (Mirisho, Neizer, & Sarfo, 2017). Although these are rough estimates, they do represent an adage often recited in the literature: infections result in low mortality, but high morbidity. As such, the health consequences of these experiences are frequently measured using disability-adjusted life years (DALYs). These years lost to illness, disability, or death are the result of both acute and chronic infections. Acute symptoms are immediately obvious and can include abdominal pain, nausea, and diarrhea. Long-term infections, on the other hand, are able to persist because they are untreated or symptoms are subclinical until later in life. Initial infections are often acquired when children first begin interacting with their wider environment (around pre-school years). It is likely that children born into extreme poverty will continuously lack access to adequate water, sanitation, and hygiene (WASH), resulting in subsequent lifelong re-infections (Weatherhead, Hotez, & Mejia, 2017). Secondary morbidities, such as cognitive

impairment or cancer can then develop, further limiting economic opportunities and perpetuating poverty in these communities (Weatherhead, Hotez, & Mejia, 2017).

This intersection between children's health and sustainable development has created a common goal for many large and well-funded organizations. Of note, the World Health Organization (WHO) and UNICEF have both committed to a goal of treating at least 75% of at-risk school-aged children via mass drug administrations (MDA). The intent of this policy is straightforward, and at a cursory glance, simple to achieve; early treatment should prevent the onset of profound morbidities. However, as they tend to do, complications arose. Administrators found that single-dose medications had low or diminished efficacy due to repeated use and polyparasitism. This led to the development of "rapid-impact" packages containing multiple drug classes to treat overlapping infections of different parasites. However, resistance has been a concern, and the expansion of novel therapies has been slow. Adding to the problem, lack of infrastructure improvement and political instability have attributed to rapid rates of re-infection (Weatherhead, Hotez, & Mejia, 2017). As Hotez et. al (2016) note, implementation of these programs requires local context, including vector control and collaboration with public leaders.

While much has improved since the commencement of global elimination efforts, there is still much more to be done. Current efforts are focused on reaching MDA goals, but this will effectively only control morbidity and have little impact on the rates of re-infections (Ziegelbauer et al., 2012). Curbing re-infection rates requires a comprehensive approach, including development of sustainable infrastructure and education on the harmful health outcomes of certain behaviors. Successful elimination requires changes to be made at both the community and individual level. Until then, recurrent infections that lead to major health consequences and hinder social and economic success will continue. Keeping in mind the overwhelming number of people burdened with infection, the accumulation of loss is hard to overstate.

This paper will focus on the long-term outcomes of diseases that result from early and recurrent nematode or Schistosoma infections. There will also be a discussion on future interventional strategies and therapeutic developments.

Roundworms (Ascariasis)

Ascariasis is the disease which results from an *Ascaris lumbricoides* infection in the small intestine. Infection follows the fecal-oral route; after the ingestion of infective eggs, larvae hatch and penetrate the duodenal wall to enter the blood stream. They then enter the pulmonary circulation, invade alveolar walls, ascend the bronchial tree to the throat, and are subsequently swallowed back into the small intestine, where they develop into adult worms. Female worms produce approximately 200,000 eggs per day, which are then passed through the feces and settle into the soil, where they develop infectivity over the next several weeks. The cycle begins again when the next person eats food or drinks water that has been contaminated. It is for this reason that regions with inadequate sanitation (and/or use "night soil" as fertilizer) are the ones that continuously secure the highest burden of this disease (The Centers for Disease Control and Prevention [CDC], 2019).

Adult worms can live up to 2 years in the small intestine and grow to be quite large (females can be up to 35 cm) (see Figure 1). Extensive worm burden can induce abdominal pain, intestinal obstruction, or perforation. While these issues are undeniably critical, they are not a typical occurrence. More commonplace is a cumulative effect leading to the development of secondary morbidities. For example, growth failure is the most well-known outcome of a long-term *Ascaris* infection. One study finds that a fecal count of 1000 eggs per gram (epg) increases the odds of stunting by 47% (Jardim-Botelho et al, 2008). However, this outcome can only be discerned over an extended period of time (Stephenson, 1999). As a result, interventions that would allow the child to regain their expected growth trajectory could be delayed.

Figure 1

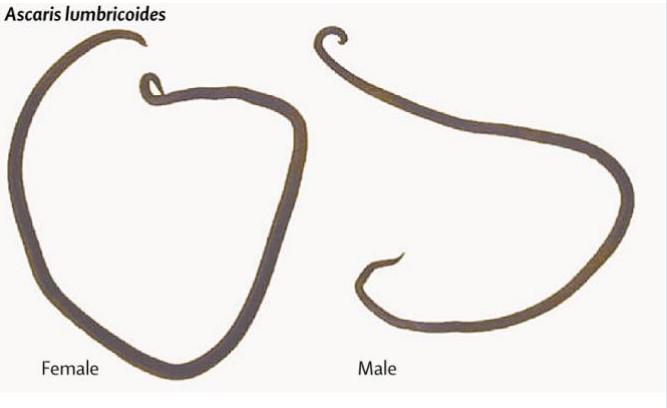

Fig 1. Adult male and female *Ascaris lumbricoides.* Adapted from Bethony et al (2006).

There are a few proposed mechanisms that explain the observation noted above. The first describes the impact of micronutrient malnutrition, which occurs due to decreased food intake or impaired nutrient absorption. The degree of diminishment of appetite is typically proportional to the intensity of infection (see Figure 2); a study of Guatemalan children with acute respiratory infections or diarrhea showed an 8 and 18% deficit in caloric intake compared to control (Stephenson, 1999). It is thought that temporary anorexia is beneficial to recovery, as it restricts the amount of nutrients being supplied to an invading pathogen. However, anorexia over prolonged illness can result in undernutrition (Plata-Salamán, 1996). An additional cause of micronutrient malnutrition is due to malabsorption. Gut helminth infections damage intestinal mucosal epithelial cells, impairing their ability to take in both macro- and micronutrients. One study found that children infected with *Ascaris* effectively absorbed only 80% of a tracer dose of vitamin A, while uninfected children absorbed 99% of the same dose (Stephenson, 1999). The compounding effect of both limited food intake and impaired absorption has shown to significantly impede growth, but it is likely not the full explanation.

Figure 2

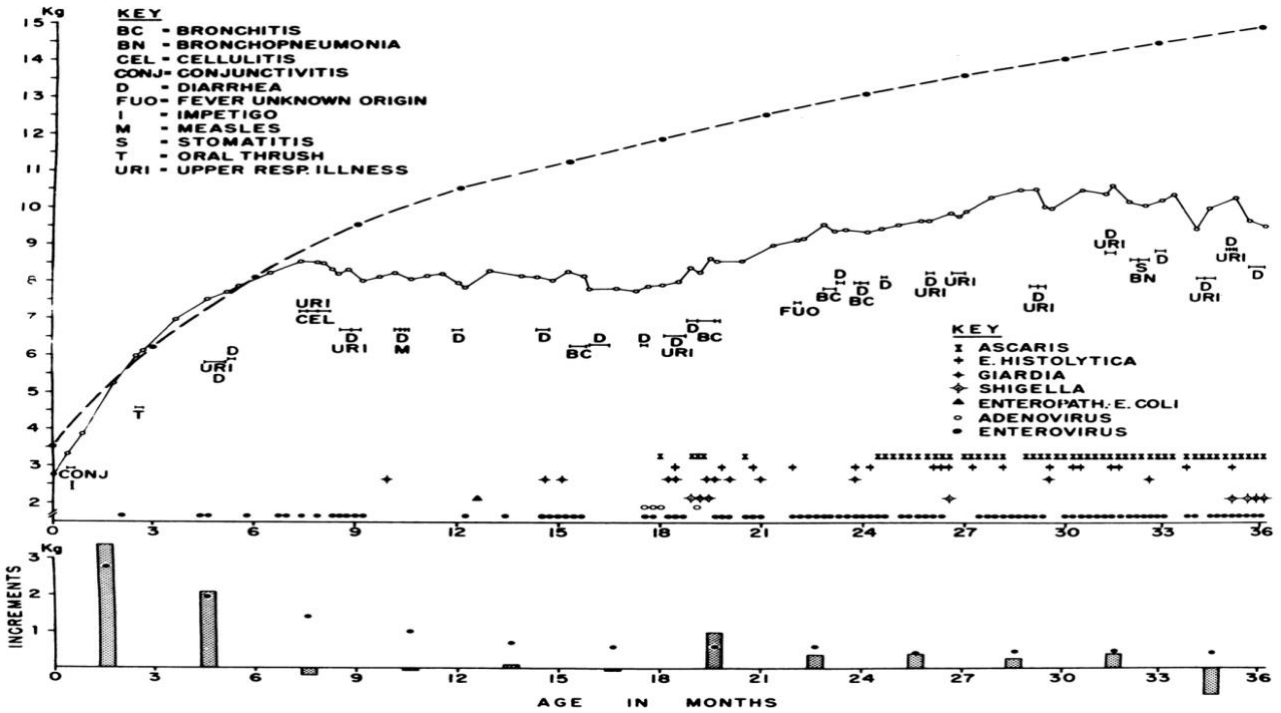

Fig 2. Weight gain of a Guatemalan child in first 3 years of life compared to expected weight gain. Weight loss was associated with repeated episodes of infection. Adequate growth seen during period of exclusive breast feeding. Adapted from Stephenson (1999).

Another proposed mechanism to explain growth failure involves the broader impact of infection on bone development. The immune response to an invading pathogen heavily relies on the production of pro-inflammatory cytokines such as TNF-α, IL-6, or IL-1β. It has been shown that these cytokines are also implicated in the generation of osteoclasts, cells that break down bone for reabsorption. Osteoclastogenesis regulation requires the interactions of three molecules of the TNF family: receptor activator of nuclear factor kappa-B (RANK), the RANK ligand, and osteoprotergerin. Importantly, IL-6 induces RANK ligand expression in osteoblastic cells, leading to apoptosis. Osteoclasts prevail, driving an increase in bone resorption (Kayamori, 2010). Bone remodeling is highly dependent on this interplay between osteoblasts and osteoclasts. Because bones grow rapidly during childhood and adolescence, this population is particularly sensitive to excessive production of osteoclasts (Lechner, Rudi, & von Baehr, 2018). Chronic or recurrent *Ascaris* infections result in a continuous immune response, leading to heightened levels of osteoclasts and impairments in long bone growth.

Current supportive therapy aims to maintain growth by reducing the severity of disease and increasing nutritional support. *Ascaris* infections can be treated with the anthelminthic medication, Albendazole. This is a broad-spectrum drug that selectively binds to parasite β-tubulin. This inhibits microtubule polymerization, disrupting cell structure as well as limiting biological processes such as the segregation of chromosomes during mitosis and glucose uptake (Abongwa, Martin, & Robertson, 2017). The parasite is essentially nonfunctional and can be passed without further issue. The development of other chemotherapeutics effective against Ascaris is ongoing, but tend to be less amenable to MDA than the benzimidazoles.

In regard to nutritional support, one study investigated the impact of iron supplementation and deworming on the growth performance of preschool Beninese children. While they did not observe a significant difference in anthropometric measurements (weight, height, mid upper arm circumference, triceps skinfold), they did note a long term (7 month) significant increase in hemoglobin. The researchers speculate that the lack of significant growth was due to high fiber/low protein diets and continuous parasitic infection (Dossa, 2001).

It has been known for some time that ascariasis and the other gut helminthic infections are associated with growth failure. Height and weight measurements are readily available. What is more difficult to evaluate has been the impact of these infections on cognition. Cognitive performance is slightly more elusive to capture but is potentially more significant in terms of schooling and breaking poverty cycles. Infected children in Turkey have shown a critical delay in language development, with a delay rate 2.2 times higher than their non-infected peers (Doni et al, 2015). Disadvantages such as these, especially during early development, tend to compound over time and negatively impact the trajectory of one's success.

Much like growth, deficits in cognition are proposed to be due to micronutrient malnutrition. Fiorentino et al (2018) studied the effect of multi-micronutrient-fortified rice on cognitive performance in Cambodian schoolchildren. In addition to the nutrient supplements, the children were also dewormed with Mebendazole, an anthelmintic belonging to the same drug class as Albendazole. All cognitive scores (measured using Raven's colored progressive matrices (RCPM), block design, and picture completion) improved six months after the implementation of the fortified diet. Zinc and iron were notably significant in the successful enhancement of cognition. Zinc has been previously implicated as an essential catalyst for a number of mammalian enzymes. Importantly, zinc deficiency has been associated with reductions in stem cell proliferation, increased neuronal precursor apoptosis, and impaired neuronal differentiation (Levenson & Morris, 2011). The other important nutrient, iron, is associated with growth failures (as noted above), as well as cognitive and emotional alterations. For example, anxiety driven behavior has been linked to poor iron status. It is thought that iron plays a role in the neurochemical circuits including the monoaminergic and gamma-aminobutyric acid (GABA) systems. Both monoamines and GABA are involved in mood regulation and neuronal activity, and for this reason, emotional behaviors are likely to be affected by iron levels (Kim & Wessling-Resnick, 2014). Like long bones, brain growth during childhood occurs rapidly, so this age group is especially sensitive to deficiencies in nutrients required for neurogenesis and neurotransmission.

Whipworms (Trichuriasis)

Like *A. lumbricoides, Trichuris trichiura*, otherwise known as the human whipworm, follows the fecal-oral infectivity route. Ingested infective eggs hatch in the small intestine where they mature and establish in the colon. Adult worms typically live in the cecum and ascending colon. Here, female adult worms deposit 3,000 to 20,000 eggs per day. These eggs are passed with the stool and can develop infectivity while in the soil (CDC, 2013). Trichuriasis, the condition of being infected with *T. trichiura*, lacks the pulmonary migration phase seen with Ascarisasis and those afflicted generally have little to no symptoms. Adults with heavy infections can experience gastrointestinal distress, diarrhea, or rectal prolapse; in children, trends similar to ascariasis, such as anemia, growth retardation, and impaired cognitive development, are noted. *T. trichiura* worms are much smaller than ascaris worms, and generally do not cause intestinal blockages (See figure 3).

Figure 3

Fig 3. Adult male and female *Trichuris trichiura* Adapted from Bethony et al (2006).

The pathology of this infection resembles inflammatory bowel disease (IBD), an inflammatory gut condition caused by an immune response to bacteria in the microflora. In the case of trichuriasis, the colitis is caused by the adult parasite attaching to the intestinal wall (Bethony et al., 2006). Attachment results in a Th2-type immune response, and prolonged inflammation damages crypt architecture and causes goblet cell hyperplasia. Parasite colonization also triggers the expansion of Tuft cells, which are taste receptors that respond to a broad range of agonists. They seem to play a role in parasite recognition, and can initiate type 2 mucosal immunity. Gut invasion is possible because *T. trichiura* secretes a protein called TT47, which forms ion-conducting pores in lipid bilayers (Drake et al., 1998). These proteins facilitate the production of the characteristic syncytial epithelial tunnels in which the worms anchor and

reside. The development of this niche is thought to help the worm counteract host peristalsis, and is unique to this parasite (Tilney et al., 2005).

In addition to eliciting inflammatory responses, it seems as though chronic trichuriasis infections can also alter the host's gut microbiota. Using a mouse model, Houlden et al. (2015) identified changes in the alpha and beta-diversity of stool as soon as 14 days post infection. This decline in diversity was especially seen in the Bacteroidetes phylum, which is essential for carbohydrate degradation into short-chain fatty acids, which can then be absorbed and used for energy. Clearance of the infection brought about recovery of a microbiota to levels similar to uninfected animals. The researchers hypothesized that the infection introduced a selective pressure for the maintenance of an "infected" microbiota, and once this is lost, the original environment can be re-introduced. However, this field is being currently investigated, as well as the interactions between parasitic infection, the microbiome, and the immune system.

Like ascariasis, the current drug of treatment for trichuriasis is Albendazole. However, the efficacy of this drug is quite low, with cure rates of a single dose ranging between 2.6% - 64.5%. Two doses significantly increased efficacy (67%-83%), but this is still smaller than what is seen in ascariasis treatment (Adegnika et al., 2015). Anti-parasitic therapies have instead been focused on the immune-controlled mechanisms of expulsion. Chronic infection has been associated with increased epithelial cell proliferation and apoptosis, both of which are controlled by the pro-inflammatory cytokine IFN-γ. Additionally, resistant animals have demonstrated up-regulated expressions of Muc2, a mucin. Mucins are the major protein components of mucus and are essential in the innate defense of the gastrointestinal tract. Treatment with Muc5ac impeded worm viability, showing a possible novel route for therapy (Klementowicz, Travis, & Grencis, 2012).

Hookworms (*Ancylostoma spp & Necator americanus*)

Similar to the other nematodes listed above, hookworms spend a significant portion of their life cycle in the soil. Eggs in the soil release free-living rhabditiform larvae, and after 5 to 10 days develop into their infective morphology. Under optimal conditions, these infective larvae can

survive 3 to 4 weeks until contact with the human host, typically the sole of a bare foot. The larvae penetrate the skin and are carried to the lungs, where, similar to *A. lumbricoides*, they ascend the bronchial tree, to the pharynx, and are subsequently swallowed. The larvae mature in the small intestine (usually the distal jejunum), where they attach and feed on the host. The most common species that contribute to human pathology are *Ancylostoma duodenale, A. ceylanicum,* and *Necator americanus* (CDC, 2019). They have a charactersitc hook-like shape, as seen in Figure 4. Heavily infected individuals can experience blood loss up to 9.0 mL/day through the direct consumption by the parasite, as well as leakage from the attachment site. The resultant iron deficiency anemia presents as extreme fatigue and weakness, and in children can lead to inhibited growth and cognitive impairments (Jourdan, 2018).

Figure 4

Fig 4. Adult male and female Hookworm
Adapted from Bethony et al (2006).

Historically, hookworm infections have been infamous in their role of bringing about the myth of the lazy southerner (in addition to pellagra and malaria). One doctor described Southern adults as, “pale and anemic… weak, tire easily, and have shortness of breath” and children as, “dull and backward at school” (Martin & Humphreys, 2006). This negatively impacted northern perceptions and has led to a persistent generalization that the South is both lazy and backward. However, even in 1905, it was known that more than 40% of the population was infected with hookworm. This led to the establishment of one of the first commissions to fight against a parasitic disease. The Rockefeller Foundation began a campaign in 1909 that pushed education, treatment, and the construction of sanitary privies. Although the campaign ended in 1914, with the prevalence of the infection at 39%, the disease did still continue to decline over the years. This is in part because of the boosted awareness, increased expectation of sanitary waste disposal, and surging shoe use. There were essentially no cases by the 1950’s, marking one of the first successful multi-organizational campaigns against a parasitic disease (Humphreys, 2009).

Ascariasis, trichuriasis, and hookworm infections are the most common worm infections worldwide. While they typically do not produce acute symptoms in adults, children are vulnerable to the micronutrient deficiencies associated with chronic or recurrent infection. As a result, infected children can then experience growth impairment or weakened cognitive performance; both of which are conditions that can easily disadvantage an already burdened region. Interventions currently include MDAs and supplemental diets.

Schistosomes

Schistosomes are trematode worms that infect both freshwater snails and humans during their life cycle. The definitive hosts (humans) shed worm eggs into water, where they hatch and release miracidia that will then infect the intermediate hosts (snails) (Figure 5). Within the snail, miracidia develop into sporocysts that undergo asexual reproduction to produce free-swimming cercariae; these infective stages remain within the water until they come into contact with and penetrate human skin. Following this infiltration, the cercariae lose their tail and become schistosomula that follow venous circulation into the liver, where they mature into adults. Male and female worms then pair and migrate to and infect varying locations, resulting in schistosomiasis (Nelwan, 2019). The form of schistosomiasis that develops depends on the species of the invading parasite and the tissues affected; intestinal schistosomiasis is associated with *S. mansoni, S. japonicum, S. mekongi,* and *S. guineensis*, while urogenital schistosomiasis seems to be caused solely by *S. haematobium*.

Interestingly, symptoms of this disease are not caused by the worms themselves, but by the body's immune reaction to their egg deposition (CDC, 2018). There is an outer layer of chitin surrounding schistosome eggs that is responsible for the release of soluble egg antigens (SEA). These products in turn initiate the host immune reaction which follows its own typical program: production of pro-inflammatory cytokines promotes the recruitment of monocytes, eosinophils, and lymphocytes, which collectively organize into a granuloma. Each egg induces its own granuloma, and while a single granuloma does not cause damage, issues arise with accumulation (Lundy & Lukacs, 2013). Chronic schistosomiases can lead to fibrosis, mechanical blockage, and destruction of anatomic structures.

Figure 5

Fig 5. *Biomphalaria glabrata*, an intermediate host for *S. mansoni*. Adapted from Lewis et al

In more detail, proinflammatory and T_H1 cytokines, like TNF-α and IFN-γ, dominate this reaction for the first 4 to 5 weeks following infection. A profile switch to a T_H2 type response occurs around 7 weeks post-infection. This change is associated with an influx of eosinophils, mast cells, and fibroblasts to the lesion, generating the cellularity of the granuloma. As such, granuloma formulation peaks between 8 and 10 weeks of infection (Lundy & Lukacs, 2013). It has been proposed that this shift from a T_H1 to T_H2 profile is protective to the host; Fallon and Dunne (1999) found that mice that were tolerized to *S. mansoni* egg antigens experienced elevated Type 1 and decreased Type 2 cytokine responses, leading to limited granuloma response, but higher mortality rates. Granulomas, therefore, are both damaging and protective. Maintaining a balance between this beneficence and maleficence is vital for both the schistosome and host survival, and Schistosomes have accomplished this through the evolution of strategies for immunomodulation.

While it seems that the pathogenesis of a schistosome infection occurs in response to the presence of SEAs, Jenkins et al. (2005) propose that immunomodulation strategies begin as early as the initial percutaneous infiltration. Regulatory mediators of the immune response in the skin, like IL-10, IL-1ra, and IL-12p40 all present when exposed to the parasite. Interestingly, it has been observed that species that migrate through the skin slowly (*S. mansoni* and *S. haematobium*) selectively favor the induction of IL-1ra and IL-10, while the faster moving *S. japonicum* induces a wide range of pro-inflammatory mediators. Beyond this early strategy, it has also been found that CD5+ B cells in schistosome-infected mice expresses the Fas ligand, enabling them to initiate apoptosis of SEA-stimulated T_H cells (Lundy & Boros, 2002). Additionally, the schistosomes themselves release PGD2, which blocks the membrane-bound prostanoid receptor 1 (DP1) expressed on epidermal Langerhans cells (LC). LCs act as antigen-presenting cells during infection, and by interrupting their ability to prime antigen-specific $CD4^+$ responses, schistosomes

effectively impede the immune response (Angeli et al., 2001). However, it is not through these immunomodulatory mechanisms alone that schistosomes are able to persist within the host.

Left untreated, these parasites have the ability to survive within the host for 3-10 years. A secondary strategy utilized by schistosomes to accomplish this involves interference with host angiogenesis (Costain, MacDonald, & Smits, 2018). Like almost every process presented so far, angiogenesis is necessary, but excess can lead to pathologies. Examples include inflammatory disease, atherosclerosis, and diabetic retinopathy. Additionally, both tumor cells and parasites are able to hijack signals that guide this process and redirect vessel growth to benefit themselves. Chief among these signals are hypoxia inducible factor (HIF)-1, vascular endothelial growth factor (VEGF) and angeiopoietin-2 (Osherov & Ben-Ami, 2016). One study supports this statement, finding that SEA from *S. mansoni* up-regulates VEGF in human endothelial cells (Loeffler et. al, 2002). Another study found similar results; viable parasite ova deposited into host cervical walls contained significantly more vascularized tissue than calcified ova (Jourdan, et al., 2011). The sequestration of a niche within the vessel allows for the infection to persist, as nutrients are delivered at a constant rate and waste is reliably moved away.

Osherov & Ben-Ami (2016) propose the use of these survival strategies as therapeutic targets; preliminary findings using a rabbit model of *Mycobacterium tuberculosis* infection have shown that bevacizumab, an anti-VEGF-A monoclonal antibody, inhibited angiogenesis and prevented abnormal blood vessel growth, improved granuloma perfusion, and decreased overall bacterial burden. This is an interesting idea and is currently explored in cancer research. In regard to schistosome infections, the current standard treatment is with the anthelminthic drug praziquantel. Although this drug has been widely used for over 40 years, its specific mode of action has yet to be elucidated, though new evidence is showing the role transient receptor potential (TRP) channels play. It has been traditionally thought that praziquantel antagonizes parasite voltage-gated calcium channels, resulting in excess levels of calcium and uncontrolled muscle contraction and paralysis (Thomas & Timson, 2018). New investigations are beginning to show that praziquantel interacts with the schistosome TRP channel, *Sm*.TRPM$_{PZQ}$. TRP channels are important for the regulation of normal parasite neuromuscular activity; it is possible that the

interaction between the drug and this target is what is causing the observed effects (Bais & Greenberg, 2020).

Urogenital Schistosomiasis (*Schistosoma haematobium)*

In the case of a *S. haematobium* infection, adult worms migrate to and lay eggs in the blood vessels surrounding the genitourinary tract (See Figure 6). Among women, this leads to a condition known as female genital schistosomiasis (FGS), which is "likely the most neglected gynecologic condition…across Sub-Saharan Africa". Estimates place the prevalence of this condition in the tens of millions. The lesions that develop from the characteristic granulomatous reaction generate symptoms including itching, pain, bleeding, and infertility (Hotez, et al., 2019). The manifestation of this form of schistosomiasis is often mistaken as a sexually transmitted infection, which then delays the appropriate treatment and allows for the perpetuation of the parasitic infection.

Figure 6

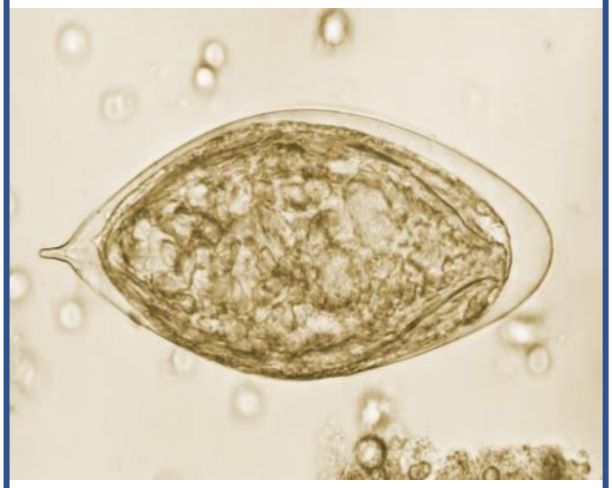

Fig 6. Photomicrograph depicting a *S. haematobium* egg. Adapted from CDC/D.S. Martin (1966)

Eventually, over prolonged infections, this damage renders the afflicted tissue dysfunctional. There is an "infertility belt" across sub-Saharan Africa, with some regions reporting rates of infertility up to 30%. While there are obvious physical health implications within this data, there is also a large social impact. In many of these communities, motherhood is central to the female gender identity and the inability to reproduce can be psychologically detrimental (Miller-Fellows et al., 2017). Although more common in women, chronic urogenital schistosomiasis can lead to infertility in both sexes. The mechanical explanation for this result has already been introduced; egg deposition into reproductive tissue prompts the arrival of granulomas, leading to the formation of lesions and subsequently, scar tissue. In this way, female infertility occurs due to fibrosis of the ovaries

or occlusions within the fallopian tubes. Male infertility can develop when there is direct testicular tissue damage or obstructions in the genital ductal system (Ribeiro et al., 2019).

Beyond mechanical means, schistosome eggs are also associated with endocrine disruptions that contribute to infertility. Along with SEA, schistosome eggs also produce catechol-estrogens, which are metabolized to active quinones by Cytochrome P450 oxygenases. The catechol estrogen-3,4-quinone can modify DNA, resulting in cancers or the downregulation of estrogen receptor (ER)-α and ER-β. Lack of Ers ultimately limits the action of estrogen, which is necessary for normal sexual and reproductive functioning. As a side note, these DNA modulators are also closely linked to carcinogenesis; *S. haematobium* is listed as a group 1 carcinogen due to its association to bladder cancer (Ribeiro et al., 2019).

Additionally, chronic FGS is associated with a three to four-fold increase in horizontal transmission of HIV/AIDS (Hotez, et al., 2019). This most likely occurs do to the inflammatory genital lesions which occur during infection, increasing transmissibility of HIV. Wall et al (2018) also note that male urogenital schistosomiasis is associated with onward HIV transmission to HIV+ partners. They suggest that in co-infected men, the increased levels of lymphocytes and eosinophils caused by the parasitic infection leads to HIV replication and increases the viral load. In support of this, one study finds that HIV binding receptors (CCR5 and CXC4) are denser on CD4 T-cell surfaces in schistosome infected men than those who are uninfected or treated with praziquantel (Secor et al., 2003). In this manner, praziquantel MDA is a possible cost-effective alternative (or supplement) for current HIV/AIDS prevention campaigns. It has also been noted that early treatment of *S. haematobium* infections can reverse the damaging effects of granulomatous lesions.

Ramarokoto et al. (2014) have shown the eosinophil granule proteins, eosinophil cationic protein (ECP) and eosinophil protein-X (EPX), to be potential markers for early-stage inflammatory lesions in women. These researchers collected urine and genital lavage samples from Malagasy women who had been diagnosed with FGS via coloscopy procedures. With this technique, they were also able to classify different types of lesions based on appearance. Rubbery papules (RP) were

identified in younger women, while homogenous sandy patches (HSP) and grainy sandy patches (GSP) were found in older women. This indicated that RP lesions were the result of recent infections and therefore more likely to contain viable eggs that were actively excreting antigens that induced granulomas dominated by eosinophils. This theory was supported by the discovery that ECP and EPX levels were indeed elevated in the lavage of women with RPs, but not those with HSPs or GSPs. It is possible that HSP and GSP lesions contained calcified eggs that no longer secreted antigen, and therefore had a reduced inflammatory response. Additionally, these later stage infections did not resolve after repeated praziquantel administrations, underlining the importance of early detection and treatment.

Intestinal Schistosomiasis (*Schistosoma mansoni* & *Schistosoma japonicum*)

S. mansoni is inarguably the most far reaching of the Schistosoma species, with prevalence in the Caribbean, South America, the Middle East, and Africa. *S. japonicum*, on the other hand, is localized to the Far East. However, both have a widespread range over time; the earliest appearance of schistosomiasis dates back more than 6000 years, with studies finding evidence of the parasite in the sediment of the skeletal remains of farmers in Tell Zeidan (ca. 5800-4000 BC) (Di Bella et al., 2018). As described above, schistosomes have evolved multiple strategies to optimize their survival within the host over this long history. In the case of severe and chronic *S. mansoni* and *S. japonicum* infections, adult worms can take up residence and deposit eggs into the portal vein, leading to a condition known as hepatosplenic schistosomiasis (HSS). Figure 7 depicts a worm blocking the mesenteric vein. Symptoms present as portal hypertension, hepatomegaly, and splenomegaly (Shaker, Samy, & Ashour, 2014).

The mechanism underlying the development of HSS is shared with FGS, in that SEAs induce immune reactions that lead to granuloma formation and subsequent fibrosis alters blood flow. While these processes are the same between the two conditions, De Cock (1986) notes that genetic factors may predispose individuals to develop HSS; one study in Brazil showed a higher incidence rate in whites compared to blacks with similar levels of infection. Further, a report from Egypt showed those with histocompatibility antigens A1 and B5 to have a greater likelihood of developing the condition. There are also a few unexpected outcomes of HSS. For example, neither

the eggs nor adult worms damage hepatocytes; despite portal vein blockages, liver perfusion is maintained due to an increased hepatic atrial flow. Additionally, there is a lack of other secondary conditions associated with chronic liver disease, like jaundice, spider nevi, and gynecomastia. Cirrhosis does not generally develop, unless there is a co-infection with another hepatotropic organism (Shaker, Samy, & Ashour, 2014).

Figure 7

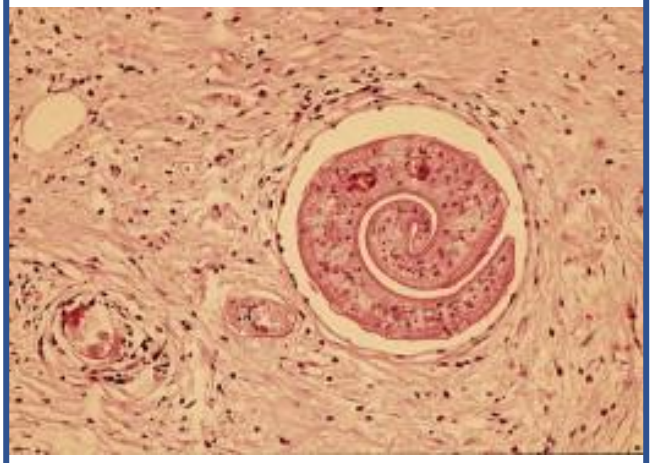

Fig 7. Adult *Schistosoma mansoni* curled up in the mesenteric vein. Adapted from Khuroo & Khuroo (2011).

Unfortunately, co-infection with hepatitis is very common. According to Andrade (1987), hepatitis B infection is 7 times more frequent in patients with HSS. *S. mansoni* in particular has been implicated in prolonged carrier states for Hepatitis B surface antigen, lengthening the period of hepatitis B virus (HBV) infectivity. This is likely due to the parasite's role in immunomodulation (described above); *S. mansoni* down-regulates Th1 cytokine responses that are important in viral infections and blocks Kupffer cells, effectively interfering with the phagocytic clearance of HBV (Ghaffar et al., 1991).

Schistosome infections can persist for many years due to the worms' immunomodulation and angiogenesis abilities. Because the symptoms of these infections tend to be tolerable, they can go unnoticed or ignored for long periods of time. However, chronicity allows for the accumulation of granulomas or fibrotic damage, leading to infertility, cancer, and hepatomegaly. Additionally, the outcomes of long-term Schistosoma infections are often associated with secondary infections of HIV or hepatitis B. Current treatment is with the anthelminthic drug praziquantel.

Other Helminthic Infections

Filarial roundworms (Lymphatic Filariasis)

Filarial worms are specialized parasites in which the definitive host is always a vertebrate (excluding fish) and the intermediate host is always an arthropod, most commonly being blood sucking insects (Janovy, Smith, & Roberts, 1996). Infected black flies or mosquitos introduce larvae onto the skin of the human host, which then enter through the bite wound and take up residence in the lymphatics. Here, they mature into adults and produce microfilariae that migrate into lymph and blood channels, infecting the next mosquito or black fly during its blood meal. Within the arthropod, the microfilariae develop into larvae. The manifestation of filariasis that occurs depends upon the location of infection, most commonly being the lymphatic system, but can also afflict the subcutaneous layer of skin or serous cavity of the abdomen. Lymphatic filariasis (LF) is caused by three worms *Wuchereria bancrofti, Brugia malayi, Brugia timori,* with *W. bancrofti* being the causative agent of an estimated 90% of cases (CDC, 2018). While this paper will generalize the contribution of all three worms to this disease, it is important to note there are variations in their transmission and disease development.

It is estimated that 15 million people suffer from lymphedema caused by LF. Many find that the condition reduces their ability to perform basic daily activities, as well as impedes their working capabilities. In an interview, one woman recounted how the condition forced her to drop out of school and depleted her income and savings. This obviously has economic implications, and especially harms those who live in poor areas where this disease is prevalent. Beyond physical hindrances, those with this disease are also prone to social isolation, sleep problems and anxiety. In rural Nigeria, there is a demonstrated need for improved knowledge and understanding of this condition, as local stigma comes from the misconception that LF has spiritual, hereditary, or sanitation origins. One study found that LF patients were 72% less likely to use bed nets, highlighting the need for greater education of the disease (Eneanya, Garske, & Donnelly, 2019).

There are two general outcomes of LF; one being a condition associated with circulating microfilaremia (MF) and the other resulting in major lymphatic compromise (See Figure 8). Although the former is generally thought of as subclinical, all those with active infection have a lymphatic abnormality, including, but not limited to, dilation or tortuosity of lymph vessels and abnormal lymph flow. The latter, which affects about 30-40% of LF afflicted individuals, has both acute and chronic manifestations. Acute presentation appears as adenolymphangitis. Chronic LF results in hydrocele, lymphedema, and in severe cases, elephantiasis (Nutman, 2013).

Figure 8

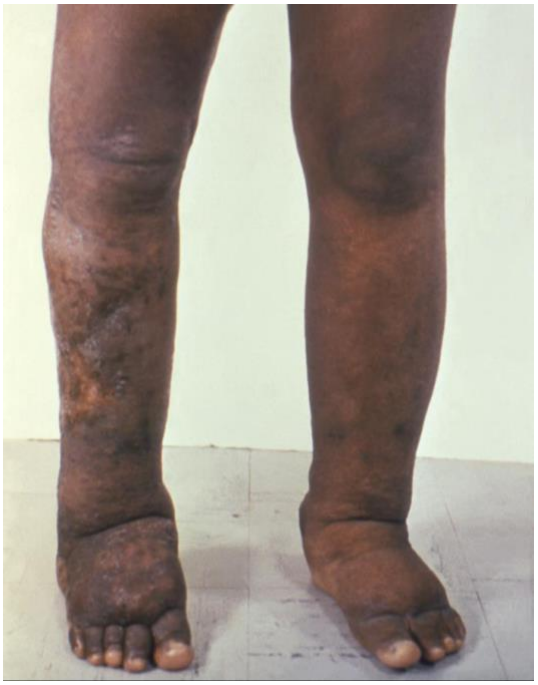

Fig 8. Patient with edema of lower legs and feet, due to a filarial infection. Anterior view.
Adapted from CDC/ R. S. Craig (1967).

The chronic and progressive nature of LF makes it difficult to recognize in its early stages, as lymphedema and other overt clinical symptoms do not typically occur in children under 10 years of age. Additionally, early MF detection was not sensitive enough to pick up on low density infections generally found in young children. As a result, they were generally left out of prevalence surveys and underrepresented. The development of filaria-specific antigen assays and ultrasound examination allowed for a greater appreciation of LF infections in children, and it is now thought that a majority of LF infections are initially acquired in childhood (Witt & Ottesen, 2001). The relevance of this early acquisition comes in regard to treatment. Once chronic pathology is established, it is irreversible even after treatment or death of the parasite (Shenoy, 2008). It follows then, that early detection is crucial for treatment and disease remission.

As mentioned above, even during the subclinical phase of the disease, there are structural and functional changes in the lymph vessels. Lymphangiectasia, or the dilation of lymph vessels, is not

restricted to the areas where adult worm nests congregate, suggesting the involvement of soluble products excreted by the parasite. As there is no inflammatory response at this point, it is likely that these excreted products act on and restructure lymphatic endothelial cells, causing dilation. Histologic examinations indeed show that these endothelial cells are preserved but aligned in different orientations (Figueredo-Silva et al, 2002). The remodeling of lymph vessels promotes stagnation of lymph and predisposes individuals to secondary bacterial or fungal infections. These further aggravate lymphatic damage, accelerating the progression of lymphedema and possibly elephantiasis (Nutman, 2013).

The disease progresses to a stage of irreversible lymphatic dysfunction upon worm death. While live worms excrete soluble products that restructure lymph vessels, the dead worm elicits the host inflammatory response and subsequent granuloma formation. One theory suggests that *Wolbachia spp.*, endosymbiotic bacteria within the nematode, have a role in inducing the host immune reaction. Dying worms release *Wolbachia* lipoproteins that are recognized by toll like receptors (TLR), which in turn stimulate both innate and adaptive immune responses (Turner et al., 2009). Interestingly, some have thought to take advantage of the symbiotic relationship between the parasite and this bacterium; anti-*Wolbachia* therapy has been proposed as a potential treatment for both LF and onchocerciasis (river blindness disease). *Wolbachia* is necessary for the growth and fertility of its nematode host. When it is cleared with Doxycycline, a very common antibiotic, the viability of the worm is affected (Wan Sulaiman et al., 2019).

The development of this therapy is still ongoing. As such, the current drug of choice for treatment is Diethylcarbamazine citrate (DEC). While its mechanism of action is not fully understood, it is thought to target the cyclooxygenase pathway and catalyze leukotriene biosynthesis. This sensitizes microfilariae to phagocytosis (McGarry, Plant, & Taylor, 2005). DEC is an effective microfilaricidal agent, but only kills around 50% of adult worms. To supplement this, DEC is often used in combination with albendazole or ivermectin (Kwarteng, Ahuno, & Akoto, 2016). Albendazole's mode of action has been previously described; ivermectin, a macrocyclic lactone, acts as an agonist to glutamate gated chloride channels. These channels are present in the neurons and pharyngeal muscles of nematodes (Abongwa, Martin, & Robertson, 2017). The

combination of DEC and albendazole was used in one study, conducted on children in Odisha, India, and showed improvement and even reversal of the disease 6 months to 2 years following administration. In order to test lymphatic flow, Tc^{99} labelled sulphur colloids were injected between the first and second toes, and images of the lymphatic system were taken using lymphoscintigraphy (see Figure 9). Images of the feet, lower limbs, and pelvis were taken at 0, 10, 30, and 60 minutes post injection. The degree of the tracer accumulation was then used to measure the severity of lymphostasis. Disease improvement was indicated by increased lymphatic flow (Kar et al., 2017).

Figure 9

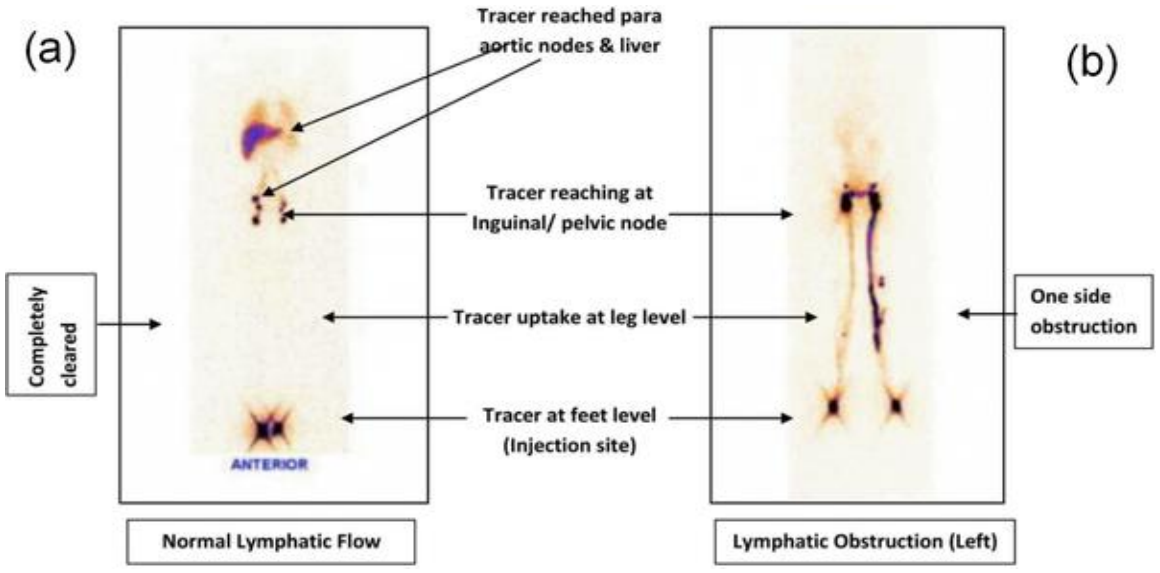

Fig 9. Lymphoscintigraphy images depicting normal lymphatic flow on the left and one-sided obstruction on the right. Adapted from Kar et al (2017).

Thailand is another example of success using DEC and albendazole MDA. Baseline surveys were initially taken in 11 endemic provinces in 2001. MDA programs were launched the following year and implemented annually until 2006 during “Filaria week” in April. The program adopted a sub-village, small population approach, ensuring better social mobilization and compliance. Child surveys were continued from 2007 to 2011 to detect ongoing infection. Children under 2 years of age, pregnant women, and those with chronic diseases were excluded. Special issues that had to be considered included zoonotic transmission of cats in the area and LF surveillance among

migrants from Myanmar. In 2017, the WHO officially acknowledged that the Ministry of Health Thailand had eliminated LF as a public health problem (Rojanapanus et al., 2019).

Interventional Strategies

Global elimination of parasitic diseases is a daunting challenge, but, as shown above, is not an entirely impossible goal. The monumental effort put forth by governmental bodies, researchers, and health care providers has begun to pay off, with five of the eight diseases originally targeted by the World Health Assembly in 1974 moving towards elimination (Reeder & Guth, 2015). MDAs have been massively critical to this success. Advancements in diagnostic technology and improvements in monitoring and surveillance techniques have also played essential roles (Hotez et al., 2016). Although certain goals have not been met yet, it would be inaccurate (and possibly unkind) to suggest that there has been no success in this area of work. However, it would be equally inaccurate (and definitely wishful) to suggest that there isn't more that can be done. At its root, this is a public health issue, and must be considered at multiple levels. This can range from individual behaviors, through therapeutic development and infrastructure building, to overall environmental control. By identifying these various targets, the appropriate interventional strategies can be determined and implanted.

Starting at the individual level, there are many behaviors that can increase one's risk of a parasitic infection. Examples include the practice of human waste as agricultural fertilizer (night soil) and the non-adherence to bed net usage. In regard to night soil, one study found that in 2010, 46% of surveyed households in Sichuan, China used it in their major crops. This spanned across the socio-economic spectrum and has contributed to the continued persistence of schistosomiasis in the area, despite improvements in sanitation infrastructure. Interestingly, the researchers also found that night soil was not used in substitution of chemical fertilizer, but in conjunction. (Carlton et al., 2015). This shows that the people do not mistrust or dislike chemical fertilizers and presents an opportunity to educate on the benefits of using it without the night soil supplementation. Taking perception into account can be important when considering individual behaviors; one study found that people in Kala-Azar endemic areas preferred light blue bed nights to green or

khaki, because they believed mosquitos were attracted to the dark colors of the forest. While preference does not translate into usage, it can still be a factor in adherence (Das et al., 2007).

A study in Myanmar looked into the rates of STH reinfection four- and six-months following MDA, and found issues in treatment non-compliance, sub-optimal drug efficacy, and other predisposed characteristics (including genetic, immunological, and environmental factors). *T. trichuris* infections were especially robust, as the Albendazole treatment is inefficacious against it (Dunn, 2019). A vaccine could bypass some of these issues, but the development of human anthelminthic vaccines has proven to be a large challenge. Hotez et al. (2016) list a few scientific limitations, including: difficulty mining parasite genomes, laboratory animal models not replicating disease, and the absence of strong correlates of protection. Despite these challenges, there are currently two hookworm antigens and three schistosome antigens in various stages of clinical trials. While these move forward, other avenues of drug development are also being explored. Baltz (2019) calls for a return to natural product drug discovery, with a focus on genome mining on culturable bacteria with large genomes. These bacteria have the capacity to encode many secondary metabolite biosynthetic gene clusters. Tyagi et al. (2019) take a different approach and are exploring the identification and use of small molecule enzyme inhibitors. Enzymes in the parasite's metabolic pathway are potential drug targets; inhibition of these "chokepoint" enzymes can cause major damage to the worm. In their report, Tyagi et al. (2019) identified three enzymes that showed the greatest potential: Pantothenate kinase (PIK), phosphodiesterase (PDE) and malate dehydrogenase (MDH).

Beyond chemotherapies and altering unfavorable individual behavior, investments in infrastructure and vector control are necessary for effective managing of re-infection rates. Insecticides have been a classic tool in vector control, but emerging resistances in mosquitoes and black fly populations require novel strategies to be formed. Current approaches are now looking into the modification of vector competence and transgenics. One design releases male insects with dominant lethality to breed with wild females, producing dead or flightless progeny (Hotez et al., 2016). Infrastructure development has also been cited as a necessary factor in controlling infection rates. However, this requires individuals from multiple disciplines, including

policymakers, scientists, and community workers to work together. This is the crux of the matter; regions most burdened with disease are also those in extreme poverty with unstable governmental bodies. War and unrest usually lead to violence which then damages infrastructure and can then produce overcrowding, sanitation issues, and malnutrition. Rudolf Virchow, known as the father of modern pathology, has famously recognized the role of government in health, “Medicine is a social science, and politics is nothing but medicine at a larger scale”. In order for the initiatives listed above to be effective, there must be support and compliance at all levels.

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