An Update on Schistosomiasis

L. Payne[1], P. L. Chiodini[1,2], A. L[1]. Bustinduy[2]

[1]University College London Hospital, London, UK, [2]London School of Hygiene and Tropical Medicine, London, UK

Contact:

Dr. Lara Payne

Email: lara.payne3@nhs.net,

Phone:+447528828777

Address: University College Hospital, 235 Euston Rd, London NW1 2BU, United Kingdom

Abstract

Human schistosomiasis is caused by trematode parasites of the genus *Schistosoma*. Four species and two distinct clinical syndromes are relevant to humans, including *S. haematobium* which is associated with urogenital schistosomiasis, whereas *S. mansoni*, *S. mekongi* and *S. japonicum* are responsible for intestinal schistosomiasis. These flukes reside in the venous plexus of the human bladder and in mesenteric veins, and release eggs, which when trapped in tissues cause inflammation and fibrosis. More specifically, *S. mansoni*, *S. mekongi* and *S. japonicum* can cause intestinal inflammation, hepatosplenic periportal fibrosis, portal hypertension and oesophageal varices that can bleed and cause death. In contrast, *S. haematobium*, a recognized carcinogenic organism, can drive bladder carcinoma and contribute to infertility and cervical cancer. Additionally, schistosomiasis can cause general anaemia, stunted growth and cognitive impairment.

Despite the chronic complications associated with schistosomiasis, public awareness in non-endemic areas is limited and travellers rarely consider risk of infection. We describe the diagnostics available in endemic areas, as well as in reference laboratories to diagnose schistosomiasis in returning travellers or migrants to resource-rich locations.

Introduction and Epidemiology

Schistosomiasis is a Neglected Tropical Disease (NTD) with over 250 million people currently estimated to be infected in 78 endemic countries [1]. Notably, schistosomiasis has the second highest mortality of any parasitic disease, exceeded only by malaria [2]. Disease control focuses on preventive anti-parasitic therapy with the praziquantel, frequently delivered through Annual, single-dose, Mass Drug Administration (MDA) programs to children > 2 years of age in endemic regions [3]. *S. mansoni* and *S. haematobium* infections primarily occur in people living in poverty in rural areas of sub-Saharan Africa. China has dramatically reduced the disease burden to near elimination with recent decades of economic development [4]; in contrast *S. japonicum* remains highly endemic across regions of the Philippines and Indonesia.

Ongoing transmission of schistosomiasis in a locality is contingent on the presence of its specific intermediate host, a particular species of aquatic freshwater snail. *S. mansoni* requires infection of Biomphalaria snails to continue its life cycle, whereas *S. haematobium* and *S. japonicum* require Bulinus and Oncomelania species snails, respectively. These snails thrive in specific ecological habitats, such as slow-moving bodies of freshwater, but have also adapted to endure periods of drought and flooding [5].

It is increasingly recognised that there is extensive zoonotic transmission of human schistosomiasis, with widespread infection of rodents and cattle in endemic regions; movement of agricultural livestock can introduce eggs which release infectious miracidia into new waterbodies and thus maintain transmission if the host-snail is present [6]. Rodents have the potential to disseminate infection over wide areas, presenting a challenge that highlights the importance of a One Health approach to disease control. Zoonotic species of schistosomes, such as *S. bovis*, have been found to cause hybrid infections in humans but the resulting disease pathology is not well established [7].

Life Cycle

Schistosomes complete a complex life cycle that is dependent on the intermediate snail host. Asexual reproduction occurs from sporocysts in the snail producing tens of thousands of infectious

cercariae that are released from the snail into freshwater and penetrate the skin of humans who are bathing or swimming in contaminated rivers and lakes. Once in human blood vessels, the schistosomulae migrate through veins to the lungs, then the liver, as they mature over 5 to 7 weeks. Male and female schistosomes mate in the mesenteric or vesical and pelvic venous plexuses and release thousands of eggs into circulation. These eggs are released in the urine and stool back into water bodies, where they hatch into miracidia to infect snails if the appropriate host species is present (Figure 1).

Pathogenesis and Immunopathology

S. mansoni and *S. japonicum* can cause liver fibrosis, portal hypertension and oesophageal varices, with pathology mediated by the host immune response [8]. Eggs become trapped in portal venules resulting in activation of Th-2 cells producing cytokines, such as IL-4, IL-5 and IL-13, leading to the formation of eosinophil rich granulomas. High levels of Th-2 cytokines provide some resistance to reinfection but are associated with increased liver fibrosis along with tumour necrosis factor (TNF)–α. Higher levels of Interferon (IFN)–γ is associated with decreased hepatic fibrosis and IL-10 is thought to be immunoregulatory.

Clinical manifestations

Early schistosomiasis.

The majority of early infections are asymptomatic except for cercarial dermatitis, which can occur when travellers are exposed to infested water and cercariae penetrate the skin, migrating to the blood vessels. Acute schistosomiasis is termed 'Katayama Fever' and is seen in immunologically naïve hosts, such as travellers visiting endemic areas. The presenting features are headache, myalgia, fever, and abdominal pain which can last for up to 2 weeks. Eosinophilia (peripheral blood eosinophils >0.45 × 10^9/L) can occur, but is not invariably found at presentation.

Chronic schistosomiasis.

Hepatosplenic schistosomiasis (HSS) is the chronic complication of advanced *S. japonicum*, *S. mansoni* or *S. mekongi* infections [9]. The clinical course is insidious with progression to liver fibrosis due to egg-induced granulomas, which eventually may lead to portal hypertension and oesophageal varices (raised liver pressure leading to dilated veins in the oesophagus that can bleed). In some cases, death may occur due to gastrointestinal bleeding from varices. *S. mansoni*, *S. japonicum* and *S. mekongi* can also cause chronic intestinal schistosomiasis with abdominal pain, diarrhoea, and rectal bleeding. The anaemia observed from intestinal schistosomiasis is multifactorial from chronic inflammation, repeated variceal bleeding, and hypersplenism due to massive splenomegaly [10]. Children often carry the highest burden of disease in endemic areas and chronic infection contributes to malnutrition and developmental delay [11] .

Schistosomiasis-associated pulmonary hypertension

Despite the substantial burden of schistosomiasis-associated pulmonary hypertension (Sch-PAH) in *S. mansoni* endemic areas, there is insufficient evidence on the mechanisms behind this process and no evidence-based guidelines for treatment. Single-centre observational studies, all conducted in Brazil, have noted a functional benefit and improved survival at 60 months in schistosomiasis induced PAH with sildenafil and an endothelin receptor antagonist, but this has not been studied or translated into clinical practice in Africa where the majority of infected individuals reside [12][13]. A systematic review found a significantly favourable haemodynamic and survival profile of Sch-PAH when compared to idiopathic PAH but relatively limited research has gone into the management of this condition and the vast majority of people living with this condition are undiagnosed and untreated [14].

Neuroschistosomiasis.

S. mansoni is associated with central nervous system pathology in an estimated 1% to 4% of infected individuals [15]. This can be classified as cerebral schistosomiasis, or spinal schistosomiasis, manifesting as encephalopathy or acute myelopathy, and is under-recognized in endemic areas with limited access to imaging [16]. Pathology suggesting neuroschistosomiasis was identified in over 50%

(16/33) of medical inpatients in Malawi presenting with unexplained, non-traumatic, spinal cord lesions [17].

Spinal cord schistosomiasis has a poor functional prognosis with only a third to a half of those afflicted completely recovering [18]. Prompt treatment with corticosteroids and praziquantel is required, but due to clinical pressures in endemic areas, and lack of awareness in non-endemic settings, there is often a significant diagnostic delay [19],[20]. Patients can present with symptoms relating to the location of eggs deposited in the brain and spinal cord; ranging from new seizures, encephalopathy, paraesthesia, focal limb weakness, alongside bladder and bowel disturbances [21].

Urogenital schistosomiasis.

S. haematobium classically migrates to the pelvic venous plexus and the venous plexus of the bladder, where schistosome eggs are shed, eventually causing calcification of the ureters and bladder. People with chronic infection report haematuria, dysuria, incontinence and abdominal pain. Severe chronic urinary tract complications of *S. haematobium* include bladder calcification and obstructive uropathies. Importantly, *S. haematobium* is a known carcinogen giving rise to squamous cell carcinoma of the bladder [22].

Female genital schistosomiasis (FGS) and Male genital schistosomiasis (MGS).

Chronic granulomas in the genital tract from *S. haematobium* infection can lead to sub-fertility, infertility, urinary tract impediments and complications in pregnancy, including spontaneous abortion, stillbirth and ectopic pregnancies. Patients with FGS or MGS frequently present with symptoms classically associated with sexually transmitted infections, which can further confound the diagnosis. FGS has been shown to increase the risk of cervical pre-cancer by six-fold and HIV infection by 3-fold, likely as *S. haematobium* eggs increase local inflammation with immune activation priming for increased transmission of HIV when T-cells are exposed to the virus [23]. The co-infection overlap is particularly concerning for Sub-Saharan Africa, where rates of *S. haematobium* and HIV prevalence are highest [24]. Males living in endemic areas experience haematospermia and sexual dysfunction

due to chronic *S. haematobium* infection which can also cause orchitis, epididymo-orchitis and prostatitis [25].

Clinical Assessment

In travellers or migrants with positive schistosomal serology, treating clinicians should evaluate the clinical signs and symptoms as possible pointers to a particular type of schistosomiasis, as the serological tests in common use are often not species-specific and there is overlap in the distribution of *S. mansoni* and *S. haematobium* in parts of Africa. Testing urine for haematuria can be used as a marker for possible urogenital schistosomiasis, particularly in migrants, where there is suspicion of active disease, but for whom the treatment history is unknown [26,27]. However, haematuria has many other possible causes. Faecal calprotectin and faecal occult blood tests can be used when there is a clinical suspicion of heavy *S. mansoni* or other intestinal schistosomal infection [28]. Although again, schistosomiasis is far from the only possible cause of abnormalities in these tests.

Ultrasound can identify hepatic fibrosis related to schistosomiasis and grade the disease severity [29]. The Niamey protocol has been standard practice for assessing clinical signs of hepatosplenic schistosomiasis by field radiographers or specialist radiologists since 1996 [30]. Features assessed to grade severity are liver texture, periportal thickening and evidence of portal hypertension. Hepatic fibrosis can be detected on ultrasound imaging prior to clinically detectable hepatomegaly. Transient elastography has been measured with the FibroScan® in endemic areas and shown to detect hepatic fibrosis due to schistosomiasis and differentiate it from cirrhosis [31]. Changes of urinary schistosomiasis described in the Niamey protocol, such as bladder calcification and hydronephrosis, have been synthesised into a Point of Care Ultrasound (POCUS) protocol named 'FASUS (Focused Assessment with Sonography for Urinary Schistosomiasis)' for use by clinicians [32].

Schistosomiasis-associated pulmonary hypertension is suspected on transthoracic echocardiography suggesting right ventricular overload, hypertrophy and increased pulmonary artery

systolic pressure. Diagnosis should be confirmed on right heart catheterization in areas where this technique is available [33].

Neuroschistosomiasis should be considered in those living or exposed in endemic regions with unexplained acute focal neurology [34]. Characteristic computed tomography (CT) and magnetic resonance imaging (MRI) changes can be observed in the brain and spinal cord consistent with egg deposition [16]. Patients should be investigated with a lumbar puncture and cerebrospinal fluid analysis for eosinophilia, schistosomal nucleic acid and Schistosoma-specific antibodies to support the diagnosis [19].

Figure 2. Clinical Diagnostic Assays

Laboratory Diagnosis

Several diagnostic methods are available, with a variety of applications, such as screening communities for parasite prevalence, diagnosis or assessing individual response to treatment (Figure 2) [35]. The availability of these assays in clinical laboratories however, varies dramatically between countries, which is in part due to whether or not they are approved for use by the local regulatory authority.

Diagnostics for schistosomiasis present multiple challenges and have different method-specific advantages and limitations. While direct visualisation of eggs in stool and urine is specific for confirming infection and species identification, there is variation in the number of eggs shed, dependent on burden of disease, and even amongst chronically infected individuals there may be no detectable eggs shed for many days [36]. In contrast, IgG ELISAs for detection of anti-schistosomal antibodies can be used to detect infection, however positive results do not distinguish between current or past infection, so it has limited utility in endemic areas, whereas negative results would not rule out infection in the recent past. Molecular testing by PCR and detection of circulating anodic antigen (CAA) in urine (see below) are superior methods for identifying active disease, but these methods are not widely available. Importantly, selection of the appropriate diagnostic tests should be determined by the specific clinical scenario; for example screening of asymptomatic people in endemic

areas, after obtaining a relevant travel history from migrants or returning travellers, with or without specific localising symptoms (Figure 2) [37]. Serology is only appropriate if there is a relevant exposure and should be tested after at least 6-12 weeks. A patient with known treated disease may require testing for CAA to give reassurance there is no longer active disease.

Microscopy.

Traditionally, in endemic areas, diagnosis has been based on faecal examination for eggs using the Kato-Katz technique for *S. mansoni* and *S. japonicum* infections, although the notable limitation of this technique is inconsistent egg production by adult worms [38]. For the Kato-Katz assay, a thick smear is prepared using approximately 40 mg of faeces sieved through nylon, then mounted under cellophane for review by light microscopy [39].

Sensitivity of direct microscopy can be improved with use of the Formalin Ethyl acetate Concentration Technique (FECT), Flotation in Centrifuge (FLOTAC) or Mini-FLOTAC techniques, with the advantage of also being able to detect co-infection with other parasites that are common in returning travellers from endemic areas [40]. Briefly, FECT requires homogenisation of 1g of stool in formalin, filtration of this suspension, addition of ethyl acetate with centrifugation, then examining the sediment layer by light microscopy. In contrast, the FLOTAC method requires formalin fixation of a 1g stool sample with filtration, followed by addition of a flotation solution to suspend helminth eggs [41]. The mini-FLOTAC assay does not require centrifugation, so is more accessible in endemic settings [42].

Detection of urinary schistosome eggs requires a terminal urine sample, ideally collected between 10:00 and 14:00 hours. The urine sedimentation method requires centrifugation of the urine at 2000g for 2 minutes, or alternatively, the urine can be left to stand for 1 hour, following which the sediment is examined under light microscopy [39].

Urine filtration is the gold standard for *S. haematobium* egg detection, but requires specific filters made of nylon, paper, or polycarbonate. The filter is examined using a light microscope on low power (x10 objective) after the addition of Lugol's Iodine to stain any eggs captured by the filter [43].

Figure 3. Schistosome eggs on light microscopy

Molecular assays.

PCR (polymerase chain reaction)-based molecular techniques have been developed to identify the presence of *S. mansoni*, *S. japonicum*, *S. haematobium* nucleic acid and hybrid schistosome infections in blood, urine, stool, and genital tract samples. Schistosome DNA can be detected by PCR in serum of patients with acute schistosomiasis (Katayama Fever) prior to the presence of eggs in stool or antibodies in serum. Importantly however, *Schistosoma* DNA may still be detectable in serum for up to 15 months after treatment, limiting its utility for assessing treatment response [44]. In female patients with FGS, infections can be detected using PCR on cervicovaginal swabs and cervical-lavage [45]. Colposcopy, both hand-held and traditional, can aid in the visual diagnosis of FGS-related lesions. (REF- FGS review from Advances) This clinical encounter is an opportunity to also screen for cervical cancer. (REF-Rafferty et al)

Schistosoma molecular assays were initially developed for academic and research use, but later deployed in clinical practice, primarily in endemic areas and reference laboratories. They have a specificity nearing 100% and sensitivity equivalent to microscopy; their primary benefit is that they can be combined as multiplex, syndromic panels to improve laboratory workflow [46]. The Novodiag® Stool Parasites Assay (Mobidiag, Espoo, Finland) is CE-IVD marked and commercially available in Europe and is reported to have 100% sensitivity for *S. mansoni* detection compared to 16% for concentrated stool microscopy with current standard practice [47]. The Stool Parasites assay simultaneously screens for 25 protozoa, helminth and microsporidia targets including *Cyclospora, E. histolytica, Taenia* and *Trichuris*. For this study, positive samples were confirmed with targeted PCR assays from reference laboratories. The wide range of pathogens assessed, and the speed and reproducibility of PCR techniques makes such methods attractive for detecting possibly unsuspected parasitic infections in migrants and travellers and distinguishing between bacterial, viral or parasitic causes of diarrhoea in a clinically relevant timescale.

As an alternative to standard PCR assays, isothermal assays; Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Assays (RPA) have also been developed, showing potential for higher amplification efficiency and improved output for large-scale, population-based screening for *S. mansoni* although this has currently only been trialled in mouse models [48].

Antigen Detection.

Specific antigens, referred to as the circulating cathodic antigens (CCAs) derived from the digestive tract of adult schistosomes, have been utilised to assist in differentiating active infection from past exposure as they are no longer detected after infection is treated. CCA detection assays have been designed for use in both serum and urine, using either a monoclonal-antibody based ELISA (enzyme linked immunosorbent assay) or lateral flow assay (LFA) based on fluorescent submicron-sized up-converting phosphor technology (UPT) reporter particles [49]. The CAA UPT-LFA has improved sensitivity compared to the CAA ELISA, so has greater utility for testing returning travellers with low worm burden infections, as it is able to detect an active infection with a single worm [50]. In human schistosomiasis challenge studies, the UPF LF-CAA was positive 6 weeks after exposure to 10 cercariae and became negative 12 weeks post treatment indicating cure [51]. A point-of-care CCA LFA (POC-CCA, Rapid Medical Diagnostics, Pretoria, South Africa) that has been evaluated using urine specimens showed sensitivity and specificity for detection of *S. mansoni* infections ranging from 52.5% to 63.2% and 57.7% to 75.6%, respectively, compared to a combined gold standard methods of faecal microscopy and an ELISA based on a soluble egg antigen [52,53]. These studies also showed that the assay is a convenient and low-cost tool for providing qualitative information on disease burden in areas of varying prevalence. Notably, false positive results have been noted to occur in non-endemic settings in infants and in those with strongly acidic urine, although this reaction can be reversed with a pH-neutral buffer [54]. The urine POC-CCA LFA is the current World Health Organization (WHO) recommended assay for assessing *S. mansoni* burden in populations to target schistosomiasis control and elimination, although its reduced specificity in children under 9 months is acknowledged [55].

Serology.

The widely used IgG ELISA based on *S. mansoni* soluble egg antigen (SEA) can detect the presence of anti-schistosome antibodies in serum, but does not differentiate between past or active infection. Schistosomal antibodies develop between 6 and 8 weeks after infection, and are highly cross-reactive between Schistosoma species. Specific ELISAs to SEA and adult worm antigen (AWA) can be used in combination to improve species-specificity [56]. A study demonstrated that relative levels of unfractionated *S. mansoni* SEA, cationic fraction of SEA and S. *margrebowiei* egg antigen (an animal schistosome similar to *S. haematobium*) allow for differentiation between *S. mansoni* and *S. haematobium* infections, however this approach is not used in clinical practice [57]. A new point of care rapid diagnostic test that utilizes bivalent antibodies on a lateral flow strip against schistosome specific IgG and IgM has been evaluated in an endemic setting, and showed sensitivity and specificity values of 95.8% and 92.4%, respectively, for diagnosis of *S. mansoni* infections in the chronic and acute phases as compared to Western Blot testing [58]. Serology can remain positive despite appropriate treatment and antibody can remain detectable for many years.

The Western Immunoblot is the assay of choice for the Centers for Disease Control and Prevention in confirming schistosomiasis in patients with positive ELISA results. Specific bands on the immunoblot can help differentiate active and past infection and can indicate the species of infecting schistosome. Commercially available kits have shown a sensitivity of 89.5% and specificity of 100% for this technique when assessing historic, parasitologically confirmed samples [58,59].

Treatment and Prevention

The WHO recommended treatment for *S. mansoni*, *S. haematobium* and *S. intercalatum* is a single oral dose of praziquantel at 40mg/kg body weight. However, higher doses (up to a total of 80 mg/kg in split doses) are needed to achieve both parasitological and antigenic cure [60]. The recommended treatment for *S. japonicum* and *S. mekongi* is 60mg/kg body weight although there are no formal trials to support this.

The need remains for a widely available test of cure, especially in lightly infected patients who may be seropositive, but who are microscopically negative for eggs in stool and urine at initial evaluation. Current assessment methods for response to therapy include monitoring for the resolution of eosinophilia, urine and stool egg-count or, where available, urine or serum CAA post-treatment [19].

Neuroschistosomiasis is managed with corticosteroids, continued for at least 2 months, to reduce the inflammatory effects on the central nervous system [18]. Concomitant *Strongyloides* infection should be excluded prior to commencing glucocorticoid therapy, but this should not delay the start of treatment as neuroschistosomiasis is a medical emergency, so initial empirical treatment with ivermectin is often necessary in that situation. An extended treatment course of praziquantel (40 to 60 mg/kg per day for 3 days) has been utilised in some centres [20]. Seizures associated with cerebral excitation due to egg associated inflammation can be managed clinically with anticonvulsants. Prevention of schistosomiasis is achieved by avoiding exposure to contaminated water bodies and travellers to endemic areas should be counselled to avoid swimming in freshwater lakes.

Summary

Schistosomiasis remains a significant public health concern despite the call for its elimination being endorsed by the WHO over 10 years ago [60]. New diagnostics have been developed with promising results, but despite the fundamental need for sensitive and specific tests to determine the presence of active clinical disease in endemic areas, many of these assays are still not commercially available, and their use is restricted to academic studies. Given the wide spectrum of clinical disease, there is a need to develop more sophisticated algorithms for diagnosis and detection of pathology attributable to human schistosomiasis in all those at risk, and especially to widen access to high quality diagnostics for those who live in endemic regions.

Figure 1. Schistosomiasis Life Cycle; (Created with BioRender.com)

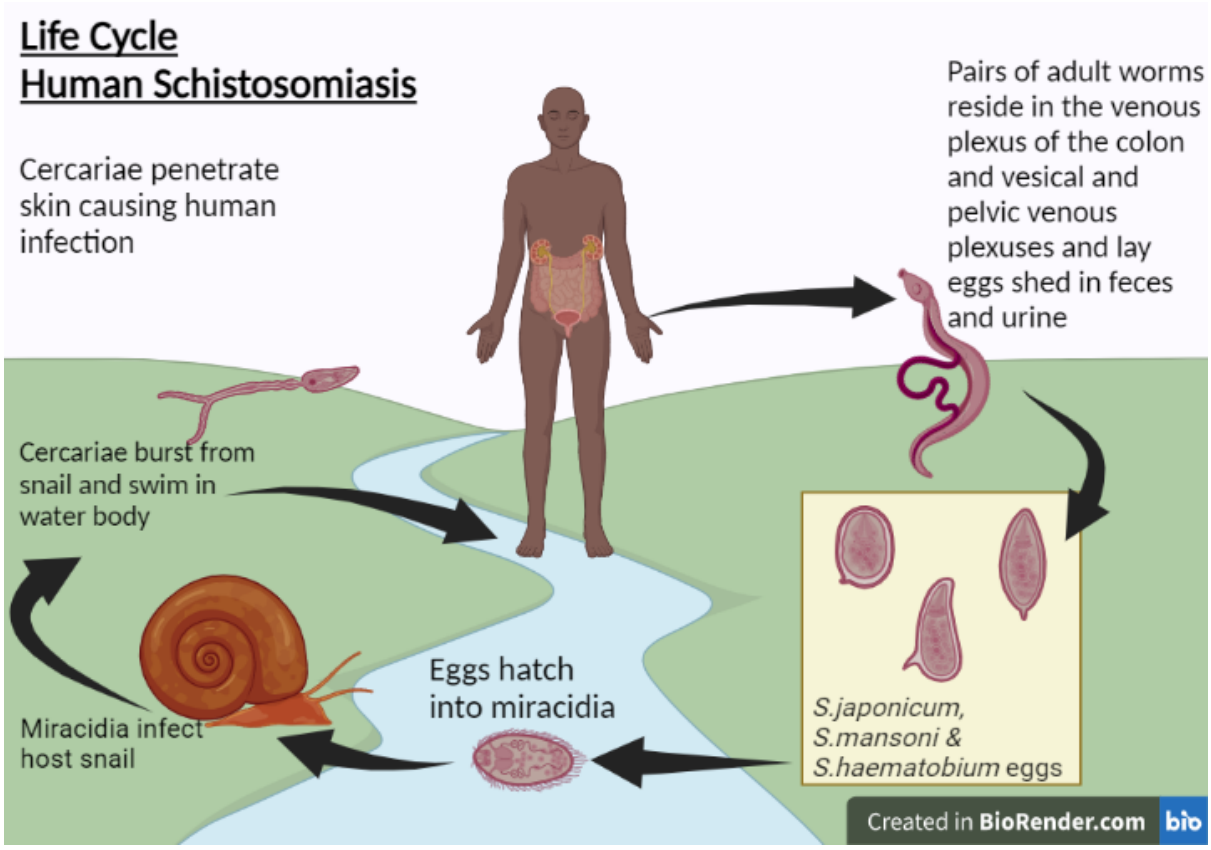
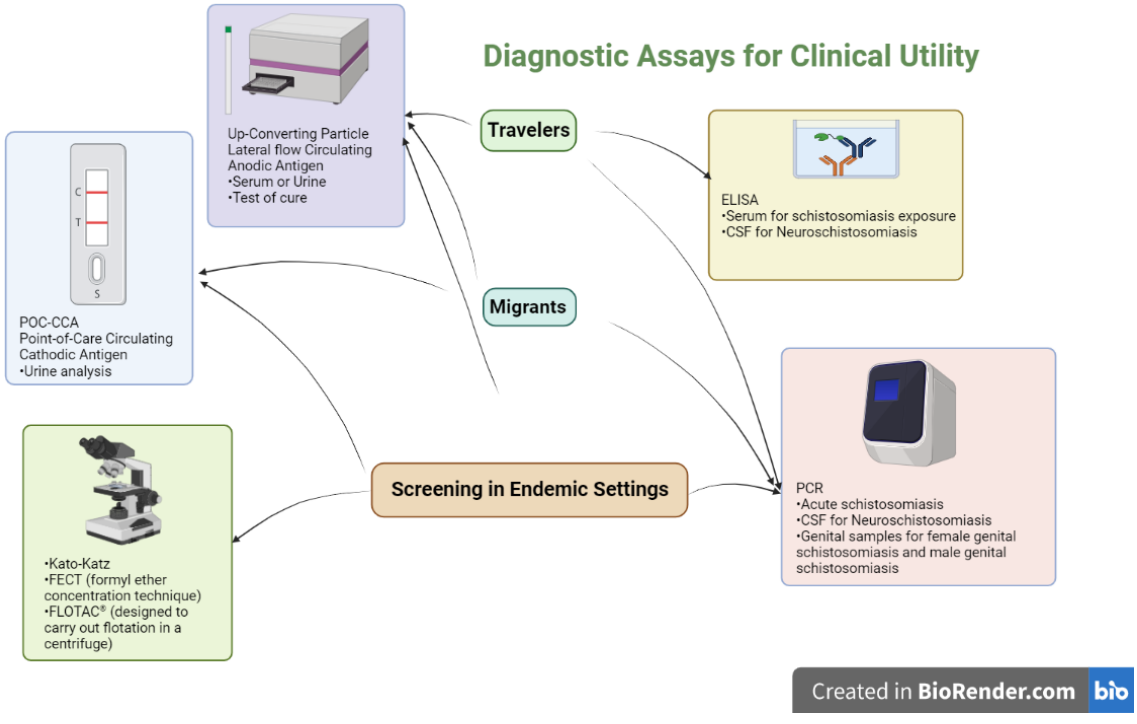


Figure 2. Clinical Diagnostic Assays (Created with BioRender.com)



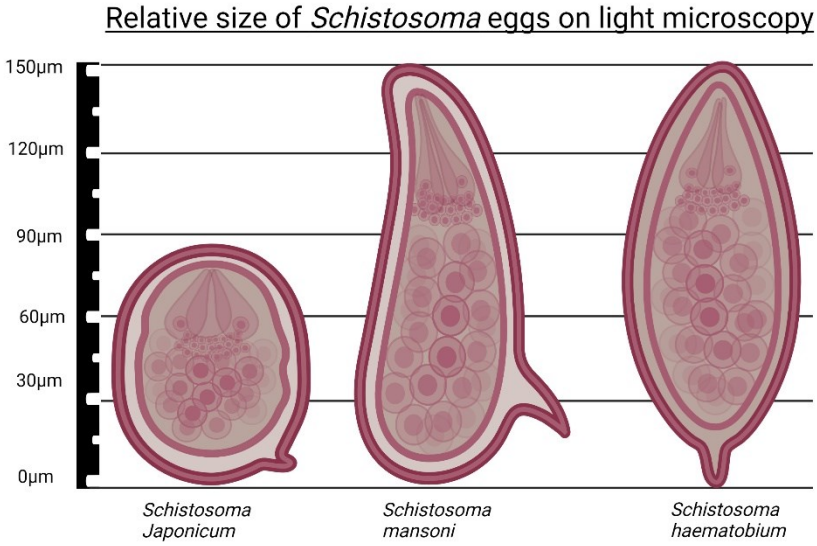


Figure 3. Schistosome eggs on light microscopy (Created with BioRender.com)

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