



# Collaborative RNAS<sup>+</sup> research: Priorities and outcomes

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## Abstract

Over the past 20 years, RNAS<sup>+</sup> has been generating research results from highly collaborative projects meant to promote and advance understanding in various aspects of schistosomiasis. Epidemiological studies in endemic countries like the Philippines, the People's Republic of China (PR China), the Lao People's Democratic Republic (Lao PDR) and Cambodia clarified the role of reservoir hosts in transmission and the use of spatio-temporal methods such as remote sensing and geographical information systems (GIS) in surveillance of schistosomiasis. Morbidity studies proposed factors that might

influence development of fibrosis, anaemia and malnutrition in schistosomiasis. Immune responses in schistosomiasis continue to be an interesting focus in research to explain possible development of resistance with age. Results of evaluation of candidate vaccine molecules are also presented. New diagnostics are continually being developed in response to the call for more sensitive and field applicable techniques that will be used for surveillance in areas nearing elimination of the disease. Several studies presented here show the insufficiency of mass drug administration (MDA) with praziquantel in eliminating the disease. Emphasis is given to an integrated control approach that can be accomplished through intensive and extensive intersectoral collaboration.



## 1. Introduction

RNAS, originally conceived to strengthen collaboration among researchers, academe and control authorities in the region and countries endemic for schistosomiasis, was later renamed RNAS<sup>+</sup> as it expanded to cover a spectrum of other target neglected tropical diseases (NTDs). The network provided critical research input that led to a better understanding of their epidemiology through intense collaboration among the national research institutes in its member countries showing that the network has achieved the critical mass needed to build capacity not only for more research but also for developing control programmes designed to meet current challenges posed by global warming, burgeoning populations and rapid socio-economic changes. This chapter deals with schistosomiasis presenting advances resulting from collaborative researches involving RNAS and RNAS<sup>+</sup> over the past 20 years.



## 2. Epidemiological studies

### 2.1 The Philippines

A dynamic model of *S. japonicum* transmission, incorporating the effects of infection intensity, age and sex of villagers in the schistosomiasis-endemic province Leyte in the Philippines, was developed by [Riley et al. \(2005\)](#). The results show that the differences in infection burden between villagers are caused by differences in transmission and infection as well as the huge potential impact of mass treatment of all humans, particularly on those with high-intensity infections and the variable effects of a sudden snail population reduction. The intensity profiles were very different in children less than 7 years old compared with older children and adults. The research group

used intensity profiles for helminths to describe the population infection status, monitor effectiveness of control programmes and provide accurate data to validate transmission models (Carabin et al., 2005a,b). It was, however, emphasized that the poor sensitivity of the Kato-Katz stool test and large heterogeneity in infection levels across villages complicate these predictions.

An extensive snail survey conducted by Madsen et al. (2008) in the same endemic province of Leyte, showed that density of the intermediate snail host in the Philippines, *Oncomelania hupensis quadrasi*, was remarkably uniform across habitats such as streams, springs, canals and swampy areas and without significant differences between rain-fed and irrigated villages, though the prevalence of infected snails showed more variability among habitats. Later, Saijuntha et al. (2014) pointed out that the phylogenetic tree and the haplotype network indicate that *O. hupensis* fragmented into *O. h. hupensis*, *O. h. formosana* and *O. h. chiui* and *O. h. quadrasi*, though the time when this occurred was not discussed. Furthermore, genetic differences between some *S. japonicum* isolates in the Philippines were shown to be genetically differentiated from the Leyte and Luzon populations, while the genetic structure of *O. h. quadrasi* populations from Leyte, Oriental Mindoro and Cagayan are significantly separated genetically. This condition has also been observed between populations of *O. h. hupensis* in mainland China, where the snail populations' genetic structure relates to geographic areas and correlates with differing shell morphologies.

In a cross-sectional study of the prevalence of intensity of infection with *S. japonicum* in 50 irrigated and rain-fed villages in Samar Province, the Philippines, Tarafder et al. (2006) showed that people who work full time in a rice farm had a higher prevalence of infection compared to those who do not. Irrigated villages were found to be 1.4 times more likely to have schistosomiasis than rain-fed villages. Results further showed that the passive treatment approach failed to control schistosome infection in this region.

Water contact was studied in detail in a five-year project, where Payne et al. (2006) used three tools in determining the frequency of water contact in four schistosomiasis endemic villages in the Philippines: (i) bimonth interviews; (ii) self-administered diaries; and (iii) field observations. It was found that the agreement between the different ways of measurement was poor leading to the conclusion that the way measurements are carried out has an important impact on studies involving water contact. This project also greatly elucidated the role of mammalian reservoir hosts in the transmission of schistosomiasis. McGarvey et al. (2006) attribute the persistence of schistosomiasis in many endemic areas in the Philippines despite

strong implementation of control programmes to the association with infected dogs and cats. This finding is backed up by [Fernandez et al. \(2007\)](#) who report that, unlike in the People's Republic of China (PR China), the prevalence of schistosomiasis in water buffalos in the Philippines is low. Rats and dogs on the other hand show higher prevalence of infections. This finding in addition to the significant village to village variation in prevalence could possibly suggest different transmission dynamics in the Philippine compared to PR China.

The results of the study of [Riley et al. \(2008\)](#) suggest that the process of acquiring mammalian *S. japonicum* infection is more important than the process of snails becoming infected and that this explains the difference in the prevalence of infection between villages. The group also showed that the contribution of water buffalos to human *S. japonicum* infection in the Philippines is less important compared to that in PR China.

[Carabin et al. \(2015\)](#), in their study of schistosomiasis in Samar, the Philippines found that dogs, cats and rats play a role in schistosomiasis transmission with dogs and rats showing consistent effects. Rats were recommended to be good sentinel animals in this environment. It was further suggested that future genetic and etiological research be conducted to understand the role of other animals in transmission. The One Health approach, involving experts in environmental sciences, veterinary and human medicine, could possibly contribute to the elimination of schistosomiasis in human communities.

[Leonardo et al. \(2012\)](#) showed lowering prevalence rates of schistosomiasis in the Philippines in a survey using stratified two-step systematic cluster sampling in a national baseline prevalence survey conducted in 2005–2008 based on one stool Kato–Katz as diagnostic tool. The group warned against lowering the guard of control authorities because of reduced rates but instead to prompt them to dig deeper to unveil the more detailed situation of the endemic areas through more intensive local surveys to identify 'hotspots' of the disease.

[Leonardo et al. \(2015\)](#) reported in detail the schistosomiasis situation in two recently discovered endemic provinces in the Philippines. It is hypothesized that the snail intermediate host has existed in these sites for some time but was only discovered now. Migration of people from endemic provinces into Gonzaga and Calatrava brought in the infection and with the presence of the snail, emergence of disease was just a matter of time. Hydrological connectivity through irrigation and river networks coursing through various geographic locations can easily transport snails into previously non-endemic areas. The last 10 years unusual weather patterns

(as gathered from interviews of locals) bringing heavy rains and floods most likely carried snails from higher to lower elevations near human habitation.

The study of [Magalhães et al. \(2014\)](#) revealed significant spatial variation in *S. japonicum* infection risk, suggesting that spatially targeted interventions like mass drug administration (MDA) could result in efficiency gains for schistosomiasis control in the Philippines. Results of the analysis show that in the three regions of Luzon, Visayas and Mindanao, males and individuals aged 20 years had significantly higher prevalence of *S. japonicum* compared with females and children <5 years. *S. japonicum* infection was widespread in the Visayas whereas it was much more focal in Luzon and Mindanao. The research group suggested that when financially possible, additional schistosomiasis surveys should be prioritized for areas identified to be at high risk but which were underrepresented in the current dataset.

The burden of polyparasitism in endemic areas of schistosomiasis in the Philippines was underscored by [Weerakoon et al. \(2018\)](#) who observed an unusually high prevalence of intestinal protozoan infections together with schistosomiasis japonica in individuals in Northern Samar, the Philippines contributing to the significant public health and socio-economic burden affecting this population. Such results further justify the implementation of integrated control strategies for intestinal parasites.

## 2.2 Cambodia and the Lao People's Democratic Republic (Lao PDR)

Results of the survey of [Matsumoto et al. \(2002\)](#) first confirmed cases of canine schistosomiasis mekongi in Cambodia proving that dogs are important animal reservoirs of *S. mekongi* in Cambodia. No schistosome eggs were found in the stools of other animals such as cows, water buffalos, pigs, horses and field rats in the five villages surveyed in Kratie Province. The study proposed that dogs could have an important role in transmission because of their high susceptibility to *S. mekongi* infection and ability to release mature eggs once infected.

The study of [Shimada et al. \(2007\)](#) confirmed for the first time the high susceptibility of *Neotricula aperta*  $\gamma$ -strain of snails from endemic areas in Cambodia to *S. mekongi* originally isolated from Lao PDR. They described three strains of *N. aperta* ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) finding the  $\alpha$ - and  $\gamma$ -strains to be common in the Mekong River, while the  $\beta$ -strain only appeared in the Mun River, a tributary of the Mekong River in Ubon Province, north-eastern Thailand. The  $\gamma$ -strain was the first strain of *N. aperta* shown to be responsible for the transmission of schistosomes in Khong, Lao PDR. Infected  $\beta$ -strain snails

have not been found in nature so far, while naturally infected  $\gamma$ -strain snails have been found in Sdau Village along the Xe Kong River, indeed the only area thus far from where *N. aperta* snails infected with *S. mekongi* have been collected outside the Mekong River basin. The discovery of high susceptibility of *N. aperta*  $\gamma$ -strain snails to *S. mekongi* from distant areas may be a crucial factor in the endemic transmission of schistosomiasis to humans.

## 2.3 PR China

Epidemiologic studies in PR China recognize the importance of knowledge and awareness and the overall socio-ecological context in understanding presence and persistence of schistosomiasis in endemic areas. In spite of the high overall awareness of the cause and preventive measures of schistosomiasis, knowledge gaps remain about the cause and transmission of the disease among students and farmers in the communities. [Zhou et al. \(2010\)](#) in their study in Eryuan County, PR China identified also other key risk factors, such as the absence of a sanitary stall house for livestock and presence of living and infected intermediate host snails in close proximity. They recommend the use of a spatially explicit Bayesian, multilevel approach to better understand the eco-social determinants that govern schistosomiasis transmission in limited geographical areas, while the study of [Yang et al. \(2013\)](#) in Jiangsu Province, PR China illustrated the usefulness of spatio-temporal analysis in assessing the risk for schistosomiasis. This approach could further be relied on with respect to surveillance and response that should increase as control progresses from morbidity control to transmission control.

The distribution of snail habitats is critical in monitoring and surveillance of schistosomiasis. [Wu et al. \(2002\)](#) showed that remote-sensing combined with ground-based ecological data collection can be used to produce classified images, not only capable of identifying snail habitats but also monitoring the impact of annual fluctuations of key environmental factors. [Zhu et al. \(2014\)](#) developed an ecological-niche model that adequately predicted the snail habitats in the mountainous regions by integration of a set of key data: the Normalized Difference Vegetation Index (NDVI), land surface temperature (LST), elevation, slope and distance from village to its nearest stream. The research group concluded that this model be used for the prediction of *O. h. hupensis* presence in mountainous areas, where snail surveys are difficult to carry out.

An important study showing the possible emergence of *S. mansoni* in PR China reassessed, using morphological and molecular delimitation procedures,

the identity and presence of *Biomphalaria* species in a southern part of PR China near Hong Kong (Habib et al., 2018). Results suggest that although *B. straminea* is the only abundant species, this still poses a potential risk of *S. mansoni* transmission in PR China, particularly in view of the increased exchange with African countries where the parasite is endemic. More studies should be conducted to understand the biology of this snail and its competitive capacity with other local snail species and to describe its range of expansion. Finally, increased surveillance of all entry ports and checking for any exotic species are recommended in order to reduce the likelihood of future introductions of potentially invasive species.



### 3. Morbidity studies

Although morbidity has generally been strongly reduced thanks to MDA using praziquantel, some studies indicate that interference with the development of morbidity is more complex than so. For example, hepatomegaly and pipe-stem liver fibrosis may continue despite intermittent chemotherapy (Wiest et al., 1992). In a subsequent study, this research group (1993) suggest that the natural history of schistosomiasis japonica in a hyperendemic community in China, namely on Jishan Island in Jiangxi Province in PR China is characterized by the persistence of hepatomegaly and schistosome-induced periportal fibrosis in adults in spite of a reduction in the prevalence of infection. In addition, Olds et al. (1996) noted that morbidity from *S. japonicum* infection appears unrelated to the intensity of infection. It is also observed to be more severe than that caused by *S. mansoni* infection in terms of liver pathology and stunting of child growth and development. Both hepatic enlargement and fibrosis can be reversed and prevented with aggressive treatment. In the Philippines, rebound morbidity after reinfection necessitates short treatment intervals between screening and treatment to ensure significant impact on morbidity. For these reasons, RNAS and RNAS<sup>+</sup> researchers have continued to study the mechanisms involved in morbidity development, anaemia in particular.

The complex nature of anaemia and the need for combined approaches to treatment in tropical settings have been made clear in a series of studies by two research groups in Leyte, the Philippines. Kanzaria et al. (2005) showed that only high-intensity infections result in significant anaemia. Their findings indicate mechanisms other than extra-corporeal iron loss that could contribute to *S. japonicum*-associated anaemia, possibly anaemia due to inflammation. This should be considered for interventions involving iron

repletion. [Friedman et al. \(2005\)](#), showing that cases with high-intensity *S. japonicum* infection were 3.5 times more likely to have faecal, occult blood, came to the same conclusion.

[Leenstra et al. \(2006a,b\)](#), on the other hand, came to the opposite conclusion. Their findings underscore anaemia as an important manifestation of chronic *S. japonicum* infection, even in low-intensity infection. This research group also assessed the impact of reinfection with *S. japonicum* after treatment with praziquantel on the mean haemoglobin level, iron-deficiency, non-iron-deficiency anaemia and inflammatory markers. It was found that the main mechanism of *S. japonicum*-associated anaemia, that is operating also in low-intensity infections, is a proinflammatory cytokine-mediated chain the eventually leads to anaemia.

Adding further information to this question, [Ezeamama et al. \(2008\)](#) showed that co-infection of hookworm and either *S. japonicum* or *Trichuris trichiura* is correlated with increased levels of anaemia than would be expected if the effects of these species had independent effects. Results imply that integrated anti-helminthic treatment programmes with simultaneous deworming for *S. japonicum* and some STH could result in greater than additive benefit for reducing anaemia in helminth-endemic regions. Taken together these studies point to anaemia as a general inflammatory response, rather than, due to extracorporeal iron loss in chronic schistosomiasis. Therefore, iron therapy may not always be the most efficacious way of ameliorating this problem.

Aside from anaemia, other subtle morbidities associated with chronic schistosomiasis are malnutrition and stunting. Results of the study of [McGarvey et al. \(1992\)](#) show that the growth retardation as an effect of high-intensity of schistosomiasis japonica in children is independent of the influence of other parasites, such as hookworm. [McGarvey et al. \(1996\)](#) also show, in a study in north-eastern Leyte that schistosomiasis caused decreased nutritional status in children, which is partly responsible for the malnutrition and reduction in growth for age described in prior cross-sectional studies. Although schistosomiasis is associated with malnutrition, the mechanisms involved are not known.

Another study in Leyte suggests that *S. japonicum*-associated nutritional morbidity could also be partly facilitated by systemic inflammation ([Coutinho et al., 2007a,b](#)). Malnutrition could therefore be the result of a combination of anorexia, metabolic changes, and body protein redistribution, leading to cachexia. This implies that nutritional supplementation alone may not be effective in lowering nutritional morbidity in areas



endemic for *S. japonicum* and should be combined with schistosomiasis control strategies.

Coutinho et al. (2005) conclude that, in children, adolescents, and young adults infected with *S. japonicum*, hepatic fibrosis is associated with impaired nutritional status. Early recognition and treatment of this type of morbidity can be critical because of the strong evidence linking malnutrition with the detrimental effects with respect to morbidity, mortality, cognitive development, reproduction and capacity for physical activity. The group further shows that even low-grade hepatic fibrosis is associated with significantly worse nutritional status. These researchers hypothesize that it could lead to malnutrition and anaemia through a systemic increase in proinflammatory cytokine levels, specifically IL-1 and IL-6—and possibly TNF- $\alpha$ .

In a prospective cohort study in Leyte, Coutinho et al. (2007a,b) found results suggesting an independent role for the Th2-biased cytokine responses to *S. japonicum* antigens in the persistence of hepatic fibrosis. Further results indicate that Th2 cytokines may play a role in the higher prevalence of fibrosis in males. Males showed consistently stronger Th2 cytokine responses to both adult worm antigen preparation (SWAP) and *Schistosoma* soluble egg antigens (SEA) than did females.

Fabre et al. (2011) suggest that the Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) is a promising biomarker for assessing risk of hepatic fibrosis in schistosomiasis and, potentially, other infectious and non-infectious causes of liver disease. With the use of a multiplex assay that quantifies predictors and effect modifiers of fibrosis (FibroPlex), these authors (2011) show that individuals with detectable tissue inhibitor of TIMP-1 had a 3.5-fold greater risk of fibrosis 1 year after praziquantel treatment. Since TIMP-1 inhibits most enzymes responsible for collagen degradation, the results indicate that schistosome-associated hepatic fibrosis could arise from excessive inhibition of collagen remodelling. Fabre et al. (2011) are presently evaluating the performance characteristic of serum measures of the TIMP-MMP axis in a bid to identify patients who may be at high risk of hepatic fibrosis.

A review done by Olveda et al. (2014a,c) shows that eggs cause bowel lesions ranging from colitis to polyp formation. In the liver, granuloma formation and subsequent fibrosis involve immune responses initially mediated by Th1 and later by Th2 lymphocytes. As a result of severe fibrosis, there may be portal hypertension-related complications causing significant illness or death. Liver biopsy and imaging techniques such as ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) can assess hepatosplenic disease for proper management. These authors also

highlight new serum markers for the evaluation of schistosome-induced hepatic fibrosis, namely hyaluronic acid, collagen type III, YKL-40 and laminin. The utility of matrix metalloproteinases and inhibitors, and cytokines needs to be further evaluated. Because of the strong correlation between the expression profile of miRNAs and the disease status/progression, circulating host miRNAs can possibly become very useful biomarkers.

Olveda et al. (2014b,c,d) claim that significant morbidity is seen in about 5% of diagnosed cases. This includes enlargement of the spleen resulting from portal hypertension caused by hepatic fibrosis. Portal hypertension can further result in massive bleeding due to ruptured oesophageal varices and pancytopenia due to hypersplenism. This research group also report a first case in the Philippines of portal vein thrombosis in a 12 year-old Filipino boy with advanced schistosomiasis. The patient was admitted because of rapidly enlarging spleen post-praziquantel treatment. After undergoing splenectomy, the patient was eventually discharged in good condition.

Further work by Olveda et al. (2017) assessed subclinical morbidity from schistosomiasis in 565 patients, using the enhanced liver fibrosis (ELF) test for the first time as a potential screening tool for disease. Results of the study showed the short-term impact of praziquantel treatment on both the prevalence and intensity of infection, but less on established morbidity. Certain measurements such as higher TIMP-1 and hyaluronic acid (HA) serum levels, and an ELF cut-off score of 8 were found to be associated with the grade of liver fibrosis. Such parameters can be used as basis of physicians in identifying individuals at greater risk of disease. The group concluded that a high level of schistosomiasis-induced morbidity is manifested by subjects with signs and symptoms suggestive of the disease in a moderately endemic area of the Philippines where active community-based chemotherapy has been implemented for over three decades.

According to Olveda et al. (2017), hepatic fibrosis, which is the root of hepatosplenic schistosomiasis, does not reverse quickly with chemotherapy. Identification and treatment of individuals with hepatosplenic schistosomiasis should be done as early as possible to prevent disease progression and possible death. Programme managers can use serum fibrosis markers, particularly high TIMP-1 and HA levels and ELF scores above 8 as basis to direct limited health care resources for schistosomiasis control. If cost can be lowered to facilitate its field application, the ELF test may assist clinicians in assessing patients with heavy intensities of infection ( $>400$  epg) and/or those with physical signs of advanced disease (e.g. MCL  $>3$ ) in determining their risk of severe liver fibrosis.

Hirose et al. (2007) show that *S. japonicum* and *S. mekongi* differ in their oviposition behaviour in their mammalian hosts which would help clarify the difference in morbidity caused by infection with these two closely related *Schistosoma* species. Their study reveals that in mice infected with *S. mekongi*, 56.6–69.4% of total eggs deposited were found in the distal small intestine 9–15 weeks after infection, while in mice infected with *S. japonicum*, 48.8–71.8% of eggs were located in the proximal small intestine during the same period. Additionally, a significantly higher number of eggs were seen in the liver in mice infected with *S. japonicum* than in those infected with *S. mekongi* which could possibly explain the more severe liver parenchymal changes in *S. japonicum* infection as detected by ultrasonography. This study also indicates that sexual maturity of *S. mekongi* lags that of *S. japonicum* which explains the higher number of mature eggs in *S. japonicum* infected mice compared with *S. mekongi*-infected mice.



## 4. Immunology and vaccine development

Noting the presence of *S. japonicum*-encoded, mammalian-like receptors for insulin, progesterone, cytokines, neuropeptides, Hu et al. (2004) propose that host hormones or endogenous parasite homologues could coordinate schistosome development and maturation. Schistosomes are also known to regulate anti-parasite immune responses through inhibitors, molecular mimicry and other evasion strategies. Transcriptome analyses, carried out by this research group (2004) based on numerous novel genes identified from a large dataset of expressed sequence tags (ESTs) generated from *S. japonicum* and *S. mansoni*, provide the basis for a thorough understanding of the molecular processes involved in schistosome nutrition and metabolism, host dependent development, immune evasion and invertebrate evolution. From this study additional potential vaccine candidates and drug targets have been proposed.

Webster et al. (1997) investigated the effects of host age and sex on human antibody isotype responses to *S. mansoni* and *S. japonicum* soluble worm antigen preparation (SWAP) and soluble egg antigen (SEA), using sera from subjects from Kenya and the Philippines. Their results show similar trends between the two populations despite differences in host, parasite and environmental differences between the two geographical locations.

A study of Acosta et al. (2002) in Leyte found that children of the 5–14 age group are the most vulnerable to rapid reinfection with females found to be more likely to be resistant. There is a reduction in the incidence in older age groups (15–19) and a lower intensity of infection starting around 15 years

of age indicating development of immunity. In a later study by [Acosta et al. \(2004\)](#), also in Leyte, results suggest that resistance may be associated with mechanisms directed towards a Th1 response, while IgE in contrast to other studies appeared to be associated with susceptibility in older individuals. It was hypothesized that in this population a Th1 response predominates, where IFN- $\gamma$  could strongly mediate protection, of particular interest being the significant Th1-type immune responses elicited by rPMY, a well-recognized vaccine candidate against schistosomiasis. This study strongly indicates the importance of IFN- $\gamma$  in protection. However, there are also indications pointing to antibodies, particularly IgG4, IgE, and IgA responses to SWAP, a preparation containing numerous potentially protective epitopes mediated by Th2-regulated responses.

The higher prevalence and intensity of schistosomiasis in younger individuals is believed to be consistent with either slow development of resistance or the requirement of host developmental changes for the expression of resistance. The study of [Kurtis et al. \(2006\)](#), also working in Leyte, discusses this age-related pattern showing that serum levels of dehydroepiandrosterone sulfate (DHEA-S), a marker for adrenal development, are elevated and associated with reduced *S. japonicum* infection and reinfection, even after adjusting for age and cumulative exposure. These results indicate that an essential property of host pubertal development facilitates resistance to infection observed in older individuals. DHEA-S has potent immunomodulatory activities, including up-regulation of Th2-driven antibody isotypes and down-regulation of proinflammatory cytokines. Whether DHEA-S facilitates resistance in humans through a direct anti-parasite effect or through an innate or acquired immune mechanisms remains unknown.

A subsequent study of [Coutinho et al. \(2007a,b\)](#) suggests that the puberty-associated rise in DHEA-S down-regulates proinflammatory immune responses thereby reducing malnutrition and anaemia in a population with high burden of chronic helminth infections. These results improve the present understanding of the complex relations between chronic helminth infections, inflammation, and nutritional morbidity which could influence other paediatric chronic inflammatory conditions. Such newly discovered regulatory mechanisms of inflammation-related nutritional morbidity further stress the importance of treating prepubescent children for helminth infections.

With respect to susceptibility of bovine reservoir hosts, [Yang et al. \(2012a,b\)](#) confirm that 'yellow cattle' are more susceptible to *S. japonicum* than the water buffalo. The disease in this type of cattle also manifests as

more serious pathological damage than in other livestock. Immunological analysis suggests that CD4<sup>+</sup> T cells might be an essential component of the immune response associated with the infection and a switch from Th1 to Th2 type polarized immunity was only clearly observed in these cows. Such results can potentially be the reason why schistosomes thrive better and cause more serious pathological impairment in ‘yellow cattle’ than in the water buffalo. The absence of a Th1/Th2 shift from in water buffalos could possibly explain the mechanism of self-cure in this animal.

Rapid reinfection that occurs after treatment, attended by extensive residual morbidity, necessitates alternative control strategies, including vaccine development. Shi et al. (2002) developed two experimental DNA vaccines capable of inducing partial protection in water buffaloes naturally exposed to *S. japonicum* infection in the field. In this study, Shi et al. (2002) proved that the vaccine candidate VRSj28 induced a 44% reduction with respect to worm recovery and a 77% reduction of the numbers of miracidia hatching from faeces, while another candidate, VRSj23, induced 33% and 68%, respectively. In this connection, Jiz et al. (2008) demonstrated that Th2-biased immune responses to paramyosin, a myofibrillar protein found only in invertebrates, are associated with resistance to reinfection with *S. japonicum* in humans. Further studies of this promising vaccine have been delayed due to challenges in the pilot-scale production of schistosome paramyosin. This molecule has been extensively investigated as a vaccine candidate for both *S. mansoni* and *S. japonicum*.

Jiz et al. (2008) developed a pilot scale expression and purification scheme for recombinant full-length *S. japonicum* paramyosin which is expected to propel studies in further evaluation of paramyosin. Wu et al. (2017) in a follow up study conducted three vaccine trials to assess the efficacy of recombinant full-length paramyosin (rSj97) in protecting water buffaloes from infection. Lower worm burdens were recorded for buffaloes immunized with the recombinant molecule indicating that rSj97 is a safe and promising molecule for use as vaccine against schistosomiasis japonica in water buffalos.



## 5. Diagnosis

Sensitive and accurate diagnostics has a vital role in reaching disease elimination goals for schistosomiasis japonica, which to this day remains a major public health and socioeconomic concern in Southeast Asia. Given the progress achieved with intense prevention and control measures, the

ability to quantify infection intensity and the use of non-invasive diagnostics are the requirements needed for effective, improved surveillance. In the Philippines, the nation-wide prevalence may be relatively low, while it can reach as high as 65% in some endemic areas. Kato-Katz stool examination (Katz et al., 1972), which is the approach used for surveys to determine prevalence, has been shown to lack the level of sensitivity needed in areas characterized as low-intensity infections.

Bergquist et al. (2016) have reviewed the diagnostic situation for the whole field of schistosomiasis, while Zhang et al. (2016) discuss the development and application of parasitological, immunodiagnostic and molecular diagnostic technology for *S. japonicum* in the PR China, also briefly mentioning diagnostic imagery, such as ultrasound and radiology. Zhang et al. (2016) observe that the diagnostic procedures used in the past may not be as suitable today, especially not in areas where control has shifted the situation to elimination and surveillance. They conclude that the diagnostic approach should be customized according to the diagnostic needs in terms of sensitivity and specificity required in a specific control stage, previously discussed for diagnostic approaches in general by Bergquist et al. (2009). In PR China, the most widely used diagnostic techniques are still stool examination by the Kato-Katz method and the hatching technique (Qiu and Xue, 1990), while immunological assays, especially antibody detection, are used for screening with the parasitological tests used to confirm diagnosis following a positive screening test.

Diagnostic tests developed to detect schistosome infection range from basic microscopic test to highly advanced molecular detection methods. Requirements for an ideal diagnostic test or set of tests include ability of the test to make an accurate diagnosis preferably performed once, cost-effectiveness in terms of labour, easy sample processing, cheap equipment and reagents. With the next goal being interruption of transmission followed by elimination, the diagnostic tool must be both highly sensitive and highly specific since the endemic areas are changing into areas characterized by low transmission, low prevalence and low intensity of infection.

In the past decades, Zhu et al. (2002) developed a rapid, simple immunodiagnostic assay for schistosomiasis based on *S. japonicum* SEA conjugated with a blue colloidal dye (D-1) produced in PR China and used to detect antibodies in the sera of schistosomiasis patients. The antigen-antibody complex was captured by anti-human IgG absorbed onto a nitrocellulose membrane dipstick by means of immunochromatography. Results using this dipstick dye immunoassay (DDIA) showed high sensitivity and good

specificity for the detection of schistosomiasis. The assay also produce rapid results, is simple and cheap and does not require advanced equipment. It proved to be more useful for screening target populations for selective chemotherapy than other immunoassays, mainly thanks the easy handling and rapid results. The reagents were also cheaper compared with the enzyme-linked immunosorbent assay (ELISA) but cross-reaction between *Schistosoma* and *Paragonimus* remains a problem of particular relevance to Southeast Asia.

Zhu et al. (2005) used the same technique to screen for *S. mekongi*. An estimated 140,000 people are at risk of schistosomiasis mekongi with 80,000 in Cambodia and 60,000 in Lao PDR (Urbani et al., 2002). The results of Zhu et al. (2005) demonstrate that *S. japonicum* and *S. mekongi* have sufficient mutual antigens to allow the use of *S. japonicum* SEA for *S. mekongi* serology. Its high sensitivity (97.1–98.6%), together with the complete specificity observed in relation to non-infected, healthy people, show that a DDIA based on SEA from *S. japonicum* can be used to screen for *S. mekongi* infections in endemic areas. However, cross-reactions with other trematode infections still need to be addressed.

In Cambodia, Kirinoki et al. (2011) assessed an indirect immunodiagnostic ELISA method using *S. mekongi* SEA. Sodium metaperiodate (SMP)-ELISA was used for the purpose of removing the glycosylated epitopes which are responsible for false positive reactions. The results of this method were compared with those using conventional ELISA. It was concluded that SMP-ELISA improved the specificity and sensitivity of schistosomiasis mekongi diagnosis, and it is expected that this method could become a powerful tool for diagnosing this disease.

van Dam et al. (2015) assessed the highly specific urine-based assays for the schistosome circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) by re-examining banked urine samples from Cambodia ( $n = 106$ ) and the Philippines ( $n = 43$ ) using the up-converted phosphor lateral flow (UCP-LF)-CAA assay and the point-of-care (POC)-CCA urine assay. Results of the evaluation showed that the sensitivity of a single urine POC-CCA was the same as that of a single Kato-Katz thick smear examination, while a combination of both CAA and CCA assays had a sensitivity close to that of triplicate Kato-Katz.

A follow-up investigation by Vonghachack et al. (2017) compared Kato-Katz stool examination, antibody detection by ELISA with the urine-based CCA and CAA assays in areas endemic for schistosomiasis mekongi. In addition, sera were also tested for CCA and CAA. The combination of applying

the CAA assay in both serum and urine produced the highest prevalence of schistosomiasis mekongi (43.2%) among the 377 participants. As expected, stool microscopy only showed 6.4% prevalence of the disease. This approach has been further investigated in both Cambodia and Lao PDR showing excellent results (Khieu et al., 2019).

The accurate estimation of the intensity of infection in the mammalian host is critical to the understanding and modelling of the transmission dynamics of schistosomiasis (Riley et al., 2005). Carabin et al. (2005a,b) determined the sensitivity and specificity of a filtration and sedimentation technique (named the DBL technique after the Danish Bilharziasis Laboratory) in various domestic and wild animals in the Philippines not only proving the technique to be valid, but also demonstrating an excellent level of sensitivity. The DBL technique was further deemed to be good for monitoring infection in animal reservoirs using at least two stool samples collected on two consecutive days.

Wu et al. (2010) examined stools from water buffaloes in Macanip, Leyte for *S. japonicum* infection by the DBL technique, the Kato-Katz technique, miracidium hatching, and a highly validated real-time polymerase chain reaction (PCR). Their results demonstrate that microscopic-based techniques intensely underrate the prevalence of *S. japonicum* infection in water buffaloes. These results necessitate the re-evaluation of the role of bovines in transmission of *S. japonicum* to humans in the Philippines. The study showed that coprologic technique seriously underrates the prevalence of *S. japonicum* in water buffaloes. Since buffaloes produce 200-fold more faeces than humans (50–60 kg/day versus 250 g/day), even those with low infection intensities can significantly contaminate the environment. Since all the eggs excreted by the water buffalo contaminate the environment, transmission to humans cannot be easily prevented.

Gordon et al. (2015) examined faecal samples from six villages in Palapag, northern Samar using the Kato-Katz method and a validated real-time PCR (qPCR) assay. In their hands, the qPCR performed significantly better than Kato-Katz in detecting schistosomiasis infection and could therefore be used in the future for surveillance and monitoring of areas where extensive schistosomiasis control has brought down prevalence and intensity infections to very low levels making elimination eventually possible. Diagnostic surveillance using molecular tools like qPCR can improve the assessment of the effectiveness and impact of integrated schistosomiasis control strategies. Countries nearing elimination of schistosomiasis can benefit much from the use of improved diagnostic techniques such as real-time qPCR and also



conventional PCR as well as the tests for circulating antigens in monitoring low-intensity infections. In PR China, where the disease is on its way to elimination, a rapid and sensitive method for monitoring the distribution of infected *O. h. hupensis* is urgently required.

Tong et al. (2015) successfully used a loop-mediated isothermal amplification (LAMP) assay targeting 28 SrDNA for the rapid and effective detection of *S. japonicum* DNA in infected and prepatent infected *O. h. hupensis* snails. This is the first report of monitoring infected snails from field sites of low endemicity areas by the LAMP assay using pooled snail samples which in turn proved to be a more efficient way to screen large numbers of samples compared with previously reported PCR and LAMP methods. The LAMP technique has since been standardized for use with collated samples comprising up to 50 snails for rapid monitoring and used as an innovative tool for surveillance and response strategies in risk areas (Qin et al., 2018).

Considering the difficulty in diagnosing schistosomiasis by egg detection and because of the superficially low parasite load after MDA in spite of the parasite transmission rates remaining high, Kato-Hayashi et al. (2015) conducted population-based studies to test the presence of cell-free schistosome DNA in endemic areas in Sorsogon province, the Philippines. Cell-free parasite DNA refers to the potential presence of fragments of parasite-derived DNA in the host's body fluids. Results show that cell-free schistosome DNA is a promising diagnostic marker to detect active schistosome infection even in cases in which the parasite ova are difficult to detect because of treatment with praziquantel such as what occurs during MDA campaigns.

While the current cost of molecular diagnostics is expectedly high, this assay can undoubtedly provide a useful tool for detecting low prevalent/intensity infections, for monitoring schistosomiasis control programmes, case detection in travellers returning from schistosome-endemic areas, and in animal and environmental surveillance of schistosomiasis. Weerakoon et al. (2017) tested a novel droplet digital polymerase chain reaction (ddPCR) assay targeting the mitochondrial gene nad1 to detect schistosomiasis japonica. The tool was proven capable of identifying both prepatent and patent infections using *S. japonicum* DNA isolated from serum, urine, salivary glands, and faeces in a murine model. Trial of the assay in a moderately endemic area in the Philippines confirmed that the ddPCR method can be a valuable new tool for the diagnosis and surveillance of schistosomiasis, particularly in low-prevalence and low-intensity areas that are already approaching elimination levels. The assay was proved to have potential for monitoring in areas where disease emergence or re-emergence is possible.

The diagnostic tools for schistosomiasis that detect eggs in stools, urine and biopsy materials from infected organs, serological techniques that detect antibodies and antigens of different stages of the parasites or schistosome DNA picked up by molecular techniques are only capable of determining presence of infection and/or intensity of infection. They are, however, incapable of measuring the severity of pathology in the target organ nor the resulting complications. Even the clinical diagnosis is difficult because of the non-specific signs of the disease. In addition, the widespread use of praziquantel is expected to result in many changes in the clinical manifestations of the disease.

The review of [Olveda et al. \(2014a,b,c,d\)](#) shows the development of several generations of imaging techniques in the last 30 years that have been used in assessment of schistosomiasis morbidity. Ultrasonography has several advantages: it is portable and can easily be brought to where the patient is, significantly lower in cost and does not use harmful ionizing radiation. In addition, real-time images are provided at the bedside, and its capacity has been improved by the Doppler sonogram which can add prognostic information in addition to routine grey-scale ultrasound. Sites that may not be visualized by ultrasound can be detected by CT and MRI. While MRI does not use ionizing radiation the cost is much higher. Techniques in the last 30 years that have been used in assessment of schistosomiasis morbidity.

[Chigusa et al. \(2006\)](#) used ultrasonography to detect the presence of thickening of the portal vein wall and formation of meandering collateral circulation of the splenic veins in four groups of people who underwent praziquantel treatment during the period 1995–2012. Ultrasound images show that thickening of the portal vein wall could potentially improve with frequent praziquantel treatments. However, praziquantel treatment had little effect on established splenomegaly with meandering collateral circulation.



## 6. Prevention and control

In spite of the major progress achieved over the past several decades in PR China, the Philippines, Indonesia as well as Lao PDR and Cambodia, schistosomiasis remains a public health problem in many places and is also emerging in some areas.

[Zhou et al. \(2010\)](#) have reviewed the epidemiological status and transmission patterns of schistosomiasis japonica in PR China where the disease is geared at elimination within a decade, particularly taking note of the

historical context and the experiences and lessons with the national control effort. It is revealed that an integrated control approach, implemented through intersectoral collaboration is critical, not only for reaching the goal of reduction of prevalence and intensity of infections but also for maintaining these parameters at low levels. There is strong emphasis on the necessity for innovation and an adequately flexible control approach to design interventions according to the changing nature and challenge of schistosomiasis control until the final state of elimination has been accomplished.

Williams et al. (2002) developed the first mathematical model on the transmission dynamics of *S. japonicum* which predicts that in the lake/marshland areas of the Yangtze River basin twice-yearly MDA is slightly better than annual MDA in reducing human prevalence. However, although praziquantel treatment in humans produces an immediate and large effect on human prevalence, bovine treatment or vaccination (blocking transmission) produces a greater impact with regard to human incidence in the longer term. Encouragingly, the model has been able to predict results of a human chemotherapy programme in Anhui Province with a high level of accuracy.

In 2000, the Philippine Department of Health implemented MDA using praziquantel as a cornerstone in the schistosomiasis elimination programme. In an epidemiological study entitled 'Schistosomiasis Transmission and Ecology in the Philippines' (STEP), aimed at measuring the effect of irrigation on infection with schistosomiasis, an eligible population of 30,187 residents in 50 villages in western Samar, was offered MDA in 2004. Tallo et al. (2008) found that this resulted in a far lower participation than expected. Existing individual and community factors were found to be related to participation but much more applied behavioural and social science research must be conducted to explain this result. Additionally, the results of the study of Ezeamama et al. (2012) to determine whether treatment of intestinal parasitic infections improves cognitive function in school-aged children, suggest that sustained deworming and simultaneous control for schistosome infections could improve children's ability to take advantage of educational opportunities in helminth-endemic regions.

Human MDA utilizing the drug praziquantel has been the mainstay of control in the Philippines for >30 years. Still, the prevalence of the disease within different endemic zones ranges from 1% to 50%. In many endemic areas, especially in remote hard-to-reach villages, severe end-organ morbidity is still present. In addition, infected children often present with subtle

morbidities such as growth retardation, malnutrition, anaemia and poor cognitive function. The significant contribution of large mammals (e.g. water buffaloes, cattle) to transmission complicates control efforts and the zoonotic nature of schistosomiasis indicates that MDA alone cannot eliminate the disease. To confirm this, [Olveda et al. \(1996\)](#) investigated the impact of annual case-finding and chemotherapy with praziquantel in the Philippines over an eight-year period. The results indicate that any sudden cessation of current control measures would lead to a rapid rebound in morbidity. It was also found that subjects chronically exposed to schistosomiasis japonica develop age-dependent acquired resistance to reinfection pointing to the possibility of vaccine development as an alternative approach for control of this parasitic infection.

[Olveda et al. \(2014a,b,c,d\)](#) conclude that the dismal results of the use of MDA for schistosomiasis control just point to the complex nature of the human–schistosome interaction. Innovative approaches that can be sustained economically should therefore be developed. [Olveda et al. \(2014a,b,c,d\)](#) implemented a combination of human mass treatment, targeted molluscicides, bovine (water buffaloes and cattle) treatment + vaccination in 22 highly endemic villages in the province of northern Samar. The vaccine (a SjCTPI-Hsp70 construct) provided a 51.2% reduction in worm burden, a 61.5% reduction in liver eggs, a 52.1% reduction in faecal eggs and hatching of faecal miracidia in a pre-clinical trial in PR China. The success of the bovine vaccine including other integrated measures may eventually lead to sustainable schistosomiasis control and elimination in the Philippines.

In a cross-sectional survey in northern Samar, [Ross et al. \(2015\)](#) found that in spite of an active schistosomiasis-control programme for >30 years that included a MDA campaign in the last 5 years, the mean prevalence of schistosomiasis among 10,435 evaluated subjects remained high at 27.1% (95% confidence interval [CI] 26.3–28.0%), and the geometric mean intensity of infection among 2832 evaluated subjects was 17.2 eggs oer gram (EPG) of faeces (95% CI 16.4–18.1). The research group concluded that MDA alone was not adequate, neither for controlling the prevalence of intensity of infection nor for sustaining a reduced morbidity of the disease. Hence sustainable control of the disease will require an integrated, inter-sectoral approach that goes beyond deworming. The authors felt that insufficient drug coverage, low cure rates, poor drug compliance and lack of baseline information already before the start of MDA made progress unattainable. In addition, adequate monitoring and evaluation must be carried out once the programme is commenced.

Leonardo et al. (2016) state that schistosomiasis elimination in the Philippines is a tall order, and that a plan is needed for elimination where all the aspects of the disease, including a budget to support proposed activities, are considered. All strategies that include WHO-recommended operational components such as water supply and sanitation, environmental management, snail control and health education in addition to chemotherapy should be implemented in an integrated and inter-sectoral manner.

In PR China, Xu et al. (2015) noted that while schistosomiasis and soil transmitted helminths (STHs) have decreased significantly as a result of integrated control strategies and socio-economic development, other helminthiasis such as clonorchiasis and echinococcosis have increased due to changes in social and environmental factors. The group recommends further studies to generate potential scientific advancements that in turn can be transformed into novel or improved interventions to accelerate the control of all helminthiasis, not only schistosomiasis. The application of chemical molluscicides remains one of the most effective measures for schistosomiasis control.

Yang et al. (2012a,b) investigated the cost-effectiveness of molluscicide application in order to find an optimal management approach to control the intermediate host snail under acceptable thresholds recommended by the national schistosomiasis control programme. The study concluded that all stages of the control/elimination programme should implement mollusciciding to maximize cost-effectiveness. The research group felt that the use of molluscicide treatment once a year at least would be needed before transition from morbidity control to transmission control. Further, environmental modification to counteract snail habitats could be done as an ultimate option during the transition stage from transmission control to transmission interruption. During the latter stage, re-emerging snail habitats must be eliminated immediately.

In a recent review of the status of schistosomiasis in PR China, Chen et al. (2018) noted that schistosomiasis control has always been conducted with strong political leadership and support at the highest level of government. The 60-year history of the control and elimination programme was initiated in the 1950s by Chairman Mao Zedong when the magnitude of the schistosomiasis problem was recognized. He led the control programme himself calling for the elimination of the disease in all the endemic 12 provinces along the Yangtze River. Sustained progress was achieved thanks to the long-term political commitment and improved further with the improving economy. The authors state that achievements of this kind can be replicated abroad provided there is long-term support from local governments

and the incorporation of social and economic approaches. The current Belt and Road Initiative project, aimed at boosting local economies with global health development through local import and appreciation of Chinese technologies and products, is expected to provide the steps necessary to achieve the ultimate goal of worldwide elimination of the NTDs, including schistosomiasis.

Ohmae et al. (2004) reported on schistosomiasis mekongi from the time it was discovered in 1957 to its present status where control efforts have been intensified. Both political and economic conditions delayed the implementation of proper control for the disease until 1983 when implementation of MDA with praziquantel rapidly brought down the prevalence of the disease. Serological diagnosis has been used to detect new but low-prevalence foci, while ultrasonography is used to evaluate remaining morbidity. The research group stresses the difficulty in controlling the disease and the need for complementing MDA with other appropriate control activities.

Muth et al. (2010) discuss the results of control activities in two endemic provinces (Stung Treng and Kratie) in Cambodia. Before 1994, baseline prevalence of schistosomiasis mekongi ranged from 73% to 88% with cases of severe morbidity like hepatosplenomegaly, puberty retardation and mortality. Starting in 1994, universal treatment with praziquantel was implemented with coverage ranging from 62% to 86% for 8 years which dramatically brought down the prevalence so that no new cases were reported in 2004 and only three cases in 2005. Mebendazole was also used to control STH infections which resulted in the reduction of ascariasis prevalence from 74.5% to 10% and hookworm infection from 86% to 40%. The report emphasizes how important political commitment is in achieving control of parasitic diseases also under conditions of minimal resources.

Muth et al. (2010) later reviewed the status of schistosomiasis mekongi in Cambodia and Lao PDR. Control programmes based on MDA with praziquantel combined with health education brought down the prevalence of the disease significantly. In Lao PDR, interventions started in 1980 but were interrupted due to political upheavals which resulted in resurgence of the disease. At present, a small number of foci continue to register high prevalence. There are still new infections in both endemic populations and travellers to these endemic areas. The review stresses the need for comprehensive guidelines for the elimination of *S. mekongi* and execution of bilateral efforts between Cambodia and Lao PDR.

Hisakane et al. (2008) evaluated the control measures against *S. mekongi* in Cambodia using mathematical models with simulations to evaluate the

effect of universal treatment and targeted mass treatment using praziquantel to reduce the prevalence. Results indicate that control of schistosomiasis mekongi in Cambodia is possible with high coverage rate of universal treatment and targeted mass treatment. A successful preventive chemotherapy administered in the entire population of two endemic regions in Cambodia dramatically reduced the prevalence of schistosomiasis mekongi from 77% in 1995 to 0.5% in 2003.

Croce et al. (2010) in their study of the cost-effectiveness of the schistosomiasis control programme in Cambodia (1995–2006) found that, while the control programme exhibited substantial economical advantages, the expense exceeded the budget given by the Cambodian Ministry of Health. It was hence proposed to solicit assistance from the international donor community to avoid reducing activities.

The paper by Khieu et al. (2019) referred to earlier is basically a diagnostic field investigation. However, some observations made refer to the current status and plans for the elimination of schistosomiasis mekongi from endemic areas in Cambodia and Lao PDR. It was shown that the elimination of schistosomiasis mekongi in Cambodia is feasible if based on highly sensitive diagnostics because of the highly focal nature of the endemic areas. In fact, *S. mekongi* has the smallest distribution of all the six different schistosome species that can infect humans.

In spite of the significant progress achieved through MDA and strong multi-sectoral collaboration, elimination remains distant with current efforts. For the situation to improve, a much stronger multi-sectoral collaboration, establishment of a common database and changing the perspective to a surveillance and response approach, which all will require increased budget allocations.

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