REVIEW ARTICLE

Vaccines and diagnostics for zoonotic schistosomiasis japonica

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SUMMARY

Schistosomiasis is one of the most prevalent, insidious and serious of the tropical parasitic diseases. Although the effective anthelmintic drug, praziquantel, is widely available and cheap, it does not protect against re-infection, drug-resistant schistosome may evolve and mass drug administration programmes based around praziquantel are probably unsustainable long term. Whereas protective anti-schistosome vaccines are not yet available, the zoonotic nature of *Schistosoma japonicum* provides a novel approach for developing a transmission-blocking veterinary vaccine in domestic animals, especially bovines, which are major reservoir hosts, being responsible for up to 90% of environmental egg contamination in China and the Philippines. However, a greater knowledge of schistosome immunology is required to understand the processes associated with anti-schistosome protective immunity and to reinforce the rationale for vaccine development against schistosomiasis japonica. Importantly as well, improved diagnostic tests, with high specificity and sensitivity, which are simple, rapid and able to diagnose light *S. japonicum* infections, are required to determine the extent of transmission interruption and the complete elimination of schistosomiasis following control efforts. This article discusses aspects of the host immune response in schistosomiasis, the current status of vaccine development against *S. japonicum* and reviews approaches for diagnosing and detecting schistosome infections in mammalian hosts.

Key words: *Schistosoma japonicum*, vaccine, diagnostics.

INTRODUCTION

Some 200 million people are infected with schistosomes in 74 countries; 120 million of these are symptomatic, with 20 million suffering severe schistosomiasis disease (Ross *et al*. 2002). The disease burden in 2010 calculated for schistosomiasis was 3 309 000 disability-adjusted life years (Murray *et al*. 2012). A meta-analysis assigned 2–15% disability weight to schistosomasis (King *et al*. 2005). Despite the availability of the effective drug praziquantel (PZQ), its relative inactivity against migratory juveniles and developing worms (Gonnert and Andrews, 1977), its inability to prevent re-infection and the possibility of resistant schistosome parasites emerging, due to years of mass administration of the drug, are important shortcomings. Schistosomiasis remains one of the most devastating tropical parasitic diseases (Colley *et al*. 2014) and imposes a high socioeconomic burden on many developing countries where schistosomiasis is endemic due to their impact on human health, particularly as these blood flukes are commonly found as co-infections with human immunodeficiency virus/acquired immunodeficiency syndrome (Kallestrup *et al*. 2006), malaria (Diallo *et al*. 2004) and tuberculosis (Elias *et al*. 2005). Accordingly, the Bill and Melinda Gates Foundation and other agencies, such as the World Health Organization (WHO), have targeted schistosomiasis, along with a number of other neglected infectious diseases, for elimination through investment in strategy evaluation, product development and operational research.

Five schistosome species, *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum* and *Schistosoma haematobium* infect humans. This article focuses mainly on *S. japonicum*, which is endemic in China, the Philippines and Indonesia and which, in addition to man, infects a range of reservoir mammalian hosts including water buffaloes, cattle, rodents, dogs, sheep and pigs. Human infection is normally acquired through activities such as fishing, bathing, farming, washing clothes and swimming as schistosomes are transmitted via freshwater containing infectious cercariae. After shedding their bifurcated tails, cercariae transform into schistosomula which locate and enter a blood vessel (more rarely a lymphatic vessel) and are carried by the flow of blood to the lungs via the pulmonary artery. After several days, the worms arrive in the hepatic portal system and further develop to adult worms (schistosomes are dioecious). The males and females pair up, mature,

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migrate downstream, and the female worms of *S. japonicum* begin egg production when the paired worms reach mucosal branches of the inferior mesenteric and superior haemorrhoidal veins. Many eggs are mainly entrapped in liver and intestines whereas others traverse the intestinal wall and are ejected in the feces. Then the eggs hatch, if they contact with fresh water, to release miracidia, which can infect amphibious *Oncomelania hupensis* snails. A miracidium forms a sporocyst which asexually produces daughter sporocysts that migrate to the snail hepatopancreas and, following another phase of asexual reproduction, release larval cercariae into fresh water.

Important biological features of *S. japonicum* are its zoonotic nature and the fact female worms can produce thousands of eggs per day, 10 times more in number than *S. mansoni* and *S. haematobium*. It is the schistosome eggs that are responsible for transmission and pathology, the latter due to the granulomas which form around the eggs trapped in the liver and other organs. Unlike the other human schistosome species, the zoonotic transmission of schistosomiasis japonica provides a novel feature that can be utilized for the development of a transmission-blocking veterinary vaccine in domestic animals, especially bovines, to help prevent human *S. japonicum* infection and resultant disease. Bovines (cattle and water buffaloes [*Bubalus bubalis*]) are the major reservoirs, contributing about 90% of the *S. japonicum* infection source in China (Chen and Lin, 2004). A study of bovines in Samar province in the Philippines in 2010 indicated a similar picture to that found in China with more than 90% of bovines infected with *S. japonicum* (Gordon *et al*. 2012). It is logical to target bovines for treatment/vaccination in schistosomiasis japonica control programmes because these animals produce considerably more feces on a daily basis than do humans. The first published mathematical model of *S. japonicum* transmission dynamics predicted that, in the lakes and marshlands of the Yangtze River basin in China, a bovine vaccine with 45% efficacy (the level of many current prototype vaccines) would reduce the endemic prevalence, but would not result in elimination (Williams *et al*. 2002). However, if accompanied by an initial period of human treatment and by improvements in human sanitation or a reduction in contaminated water contact by humans, elimination would be possible (Williams *et al*. 2002). Owing to the fact that water buffaloes are responsible for much of the schistosomiasis transmission in the marshland areas of China (Guo *et al*. 2006), a vaccine with 48–52% efficacy targeting these bovines, used in conjunction with PZQ treatment, could lead to a significant reduction in transmission with the predicted equilibrium prevalence reduced to zero after 5 years (Da'dara *et al*. 2008). Despite a number of research publications, knowledge of schistosome immunology in mammalian hosts is still limited, but this is critical to further understand the mechanism of pathogenesis in schistosomiasis and the processes associated with protective immunity in order to reinforce the rationale for successful vaccine development. Improved diagnostic tests for schistosomiasis are also required that can build on a better understanding of the immune response to schistosomes so that light infections can be identified with high specificity and sensitivity so as to determine the extent of the interruption of transmission and the elimination of schistosomiasis in a particular area. Here we review prospects for the development of vaccines and new diagnostics for zoonotic schistosomiasis.

THE HOST IMMUNE RESPONSE TO SCHISTOSOMES

The immunobiology of schistosomiasis includes the nature of the host innate and adaptive response to the schistosome parasites, knowledge of which is built on infection and immunization (with schistosome extracts or defined antigens) studies mainly in mice (including wild type and gene knockouts) but also in non-human primates and other mammalian hosts.

As reviewed by Mo *et al*. (2014), the immune response towards schistosomes, comprises 2 separate components: (1) immunopathogenesis and/or immunoregulation, which results from released antigens from eggs trapped in tissues. This leads to fibrosis and granuloma formation, collagen deposition and, in the case of *S. japonicum* and *S. mansoni*, severe hepatic periportal fibrosis, morbidity chronic inflammation and anaemia; and (2) age-dependent concomitant immunity against re-infection resulting over time from repeated natural adult worm death leading to the development of partially protective natural immunity in areas endemic for schistosomiasis. The protective effect of PZQ is thought, in part, to be due to the immunity induced against the drug-killed adult schistosomes (Mo *et al*. 2014). This partial protection has been associated with increased eosinophilia, $CD23^{+}$ B cells, interleukin (IL)-5 and anti-adult worm antigen (AWA) immunoglobulin (Ig)E antibodies together with low levels of IgG4 antibodies against these worm components (Mo *et al*. 2014).

A better understanding of innate immunity to schistosomiasis is necessary in developing strategies to protect hosts from infection or restrict immunopathology. Schistosomiasis results in a range of morbidities, most of which are not caused by the adult worms. Instead they are associated with the T-cell-dependent immune response of the mammalian host induced by schistosome eggs trapped in tissues and granuloma formation and related pathologies in target organs – mainly the liver and intestine with *S. japonicum* infection. The main immunopathology of schistosomiasis is induced by molecules secreted by the eggs, resulting in a marked CD4+ T-cell-mediated granulomatous inflammation

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involving monocytes, eosinophils and lymphocytes, likened to a form of delayed type hypersensitivity (McManus and Loukas, 2008).

Schistosoma blood flukes depend on signals from host CD4+ T cells for their growth and maturation in the mammalian host by inducing T-helper 2 (Th2)-biased inflammatory granulomas (Riner *et al*. 2013). While B cells suppress granulomatous pathology in schistosomiasis, it remains unclear whether these cells effect schistosome maturation, reproduction and granuloma development without the aid of CD4(+) T lymphocytes (Tang *et al*. 2013). However, it has been shown that T and B cells play a crucial role in both generating protection and exacerbating disease outcomes by orchestrating the immune response during *S. japonicum* infection in rodent models showing resistance to the parasite (Hu *et al*. 2012). Following cercarial infection, the early immune response is predominantly Th1, targeted at the adult worm. After egg deposition in tissues, the Th2 response becomes prominent, suggesting that egg antigens may directly inhibit the Th1 response (Liang *et al*. 2012). In general, recent investigations have demonstrated that T-cell-mediated immunity is necessary to promote acquired resistance to schistosomes in the murine model, a process mediated by activated macrophages (AAMs). Furthermore, cytokine studies also suggest that a schistosome vaccine that can induce AAMs to produce Th1 cytokines [gamma interferon (IFN-γ) and IL-2] may be useful in preventing disease (McManus and Loukas, 2008). It has been shown that IFN-γ and IgG2 antibodies, characteristic of Th1 responses and cytotoxicity, correlate with the high level of protection induced by an irradiated *S. japonicum* cercarial vaccine in pigs (Tian *et al*. 2010).

The CD4+ Th2 cellular response against schistosome egg antigens coordinates the development of granulomas which comprise cells (eosinophils, CD4+ T cells and macrophages) and collagen fibres around individual eggs (Pearce and MacDonald, 2002) (Fig. 1). Dendritic cells (DCs) can activate naive CD4+ T cells during their migration to lymphoid tissues, and acquire egg antigens, thereby inducing a Th2 response. Toll-like receptor 2 (TLR2), present at the DC cell surface, influences their maturation by inducing IL-10-secreting regulatory T cells (Kane *et al*. 2004) (Fig. 1). However, exposure of egg antigens to DCs does not stimulate them to synthesize IL-12 or co-stimulatory molecules such as CD80, CD86 or CD40. Generation of the Th2 response depends on IL-4 from an alternative source to the DC. IL-4 limits the Th1 response and acts as a growth factor to increase the Th2 response. IL-10 produced by B cells and DCs induces regulatory T-cell activity, with the potential to suppress the Th1 cell response to helminth worm-derived antigens, thereby ensuring Th2 cell polarization (McKee and Pearce, 2004). IL-10 may have a role in suppressing IL-12 production and minimizing the progression of the Th1 response.

The roles of TLR2–MHC class II, CD40–CD154 and OX40L–OX40 are important in the generation of Th2 responses to schistosome antigens (de Jong *et al*. 2002). An important feature of this Th2 immunity is the induction of alternatively AAM populations, which is crucial in regulating the pathology and worm expulsion that is essential for the host surviving schistosomiasis (Horsnell and Brombacher, 2010). IL-13 is the main Th2 cytokine that is responsible for fibrosis (Fallon *et al*. 2000) and a series of recent studies have elucidated that IL-13 is able to promote fibrogenesis (Modolell *et al*. 1995; Hesse *et al*. 2000, *2001*). IL-13 and its receptor complex have been identified as important regulators in controlling the progression of schistosomiasis (Mentink-Kane and Wynn, 2004) suggesting the possibility of IL-13-blocking therapies for the disease. A schistosome egg glycoprotein is able to induce the release from basophils of IL-4 and IL-13 by non-specifically binding and cross-linking cell-surface IgE (Schramm *et al*. 2003). The fibrogenic role of IL-13, together with IL-4, is essential for upregulating the expression of arginase in macrophages (Hesse *et al*. 2001). Arginase metabolizes L-arginine to produce proline which is necessary for the formation of collagen and fibrosis development. Th1 response mediators [nitric oxide (NO), tumour necrosis factor (TNF), IFN-γ and IL-12] can inhibit the Th2 response and induce macrophages to express, rather than arginase, inducible nitric oxide synthase (iNOS) which uses arginine for the production of citrulline and NO. During this process, L-hydroxyarginine is produced which inhibits arginase and reduces the level of expressed proline, thereby reducing collagen synthesis. The Th2 response stimulates massive blood and bone eosinophilia, increased levels of serum IL-5, and the egg-induced granuloma formation which results in collagen being deposited, tissue fibrosis and the manifestations associated with schistosomiasis (Wilson *et al*. 2007). The resolution of any helminth infection is generally correlated with a Th2 immune response by the mammalian host. Recent research has shown that schistosome worms also stimulate functional type 2 responses; for example, a parasite cysteine protease is an inducer of Th2 responses at the early stage of schistosome infection (de Oliveira Fraga *et al*. 2010).

Natural killer T (NKT) cells are activated to proliferate by glycolipids present in both schistosome eggs and worms (Zaccone *et al*. 2003). NKT cells may also play roles in modulating the classical T-cell response, accompanied by the upregulation of CD4 and downregulation of CD94 expression in infected mesenteric lymph node natural killer (NK) cells and enhanced expression of IL-4 and IL-17 in both the NK and NKT cells of infected mice (Luo *et al*. 2013). NKT cells, mast cells and eosinophils are all potential

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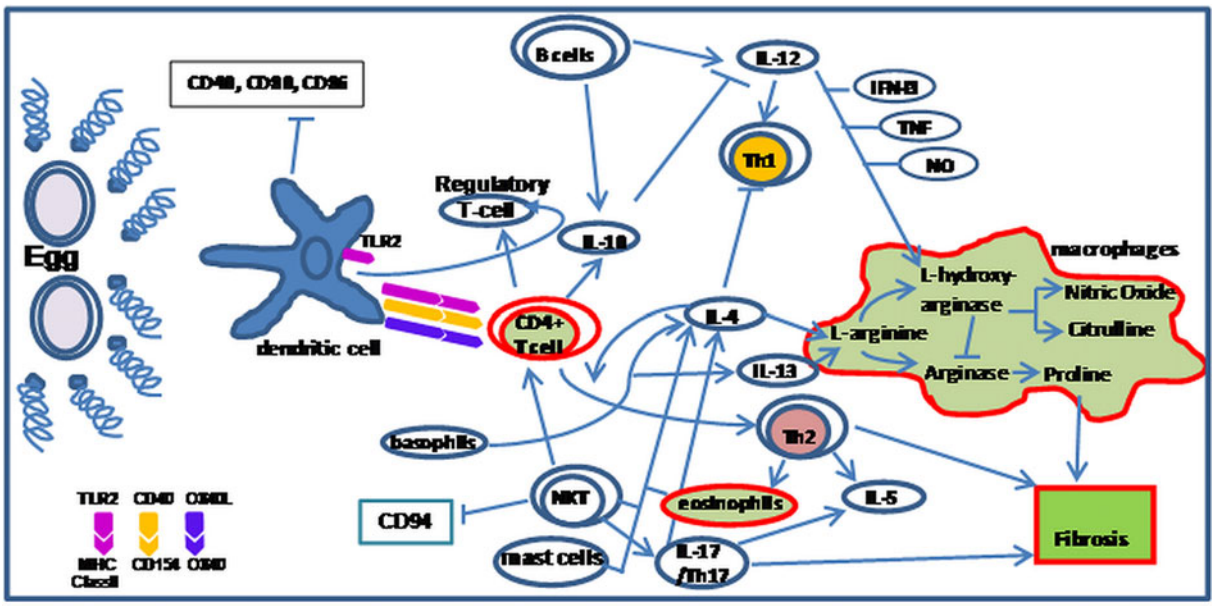

Fig. 1. Predicted model of the Th2 immune response induced by schistosome egg antigens. Granulomatous lesions comprise collagen fibres and host cells (macrophages, eosinophils and CD4+ T cells, coloured in green) around the egg. DCs can induce Th2 responses by activating naive CD4+ T cells. TLR2 located at the surface of DCs can be activated by egg-secreted proteins that influence their functional maturation by inducing regulatory T cells to secrete IL-10 (Kane *et al*. 2004). IL-10 so generated may suppress IL-12 production, which is a potent inhibitor of Th2 responses, and minimizes the progression of the Th1 response. The interactions of CD40–CD154 and OX40L–OX40 are also important in the induction of Th2 responses to schistosome antigens (de Jong *et al*. 2002). However, the exposure of DCs to egg antigens does not stimulate their expression of the co-stimulatory molecules CD40, CD80 or CD86. Induction of alternatively activated macrophage populations is a dominant characteristic of Th2 immunity. Development of the Th2 response depends on IL-4 from a source other than DCs and the main Th2 cytokine responsible for fibrosis is IL-13 (Fallon *et al*. 2000). A schistosome egg glycoprotein can induce the release of IL-4 and IL-13 from basophils and the fibrogenic role of IL-13 seems to be important, together with IL-4, to induce the expression of arginase in macrophages (Hesse *et al*. 2001). Arginase uses L-arginine to make proline which is an essential amino acid associated with collagen production and fibrosis. Mediators that are involved in Th1 responses, such as IFN-γ, IL-12, TNF and NO can hamper Th2 response development and also stimulate the expression by macrophages of inducible iNOS which uses arginine for the production of citrulline and NO. During this process, L-hydroxyarginine is produced which inhibits arginase and reduces the level of expressed proline, thereby reducing collagen synthesis. The Th2 response results in an increase in the level of serum IL-5, tissue fibrosis accompanied by massive blood and bone eosinophilia. NKT cells, eosinophils and mast cells are all potential sources of IL-4 (Sabin *et al*. 1996). As a signature cytokine of Th17 cells, IL-17, induced by *S. japonicum* infection in mouse pulmonary lymphocytes, contributes to granuloma formation and fibrosis in the liver (Chen *et al*. 2013*a*). Th17 cells express more IL-4 and IL-5 than IFN-γ, but less IL-10 (Luo *et al*. 2012; Chen *et al*. 2013*b*).

sources of IL-4 (Sabin *et al*. 1996). A large amount of IL-17 induced by *S. japonicum* infection in mouse pulmonary lymphocytes contributes to granulomatous inflammatory and fibrosing reactions in the liver (Chen *et al*. 2013*a*). However, IL-17 is a signature cytokine of Th17 cells and has been implicated in the induction of chronic inflammatory diseases (Zhang *et al*. 2012*b*). Severe hepatic granulomatous inflammation is associated with high levels of IL-17 (Zhang *et al*. 2012*b*) and lower IL-17 levels may result in favourable host-protective responses (Wen *et al*. 2011). Th17 cells are able to express more IL-4 and IL-5 than IFN-γ, but not IL-10 (Luo *et al*. 2012; Chen *et al*. 2013*b*). It has been suggested that activated NK cells in the liver can downregulate egg-induced liver fibrosis by producing IFN-γ and killing activated hepatic stellate cells (Hou *et al*. 2012). It is well established that IFN-γ is essential for the development of acquired resistance against murine schistosomiasis although recent evidence suggests that IFN-γ is not always a positive regulator of immune responses. In IFN-γ knockout mice, the disruption of IFN-γ signalling may upregulate the cytotoxic T-cell-mediated immune responses to *S. japonicum* infection (Du *et al*. 2011). However, studies on cytokine-deficient and B-cell-deficient mice demonstrated that successful anti-schistosome vaccination required the induction of strong Th1 and Th2 responses (McManus *et al*. 2010). Further research has also shown that a balance between Th1, Th17 and Th2 cytokines is required for effective schistosome larval elimination in the mouse model (Tallima *et al*. 2009; El Ridi *et al*. 2010).

It should be emphasized that the mechanisms of immune responses to schistosome infections in the mice cannot be completely generalized to humans or

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other natural hosts (Lebens *et al*. 2004). Clinical vaccine development against schistosome infection has been hampered by a limited understanding of the mechanisms of protective immunity in humans. As reviewed by Siddiqui *et al*. (2011), factors predictive of resistance in humans from a number of immune-epidemiological studies include a high concentration of serum parasite-specific IgE, increased circulating CD23 + B cells, eosinophilia and secretion of IL-5 in response to crude worm extracts. However, whether any of these immune responses is directly involved in worm killing has not been elucidated. As arguably the most relevant non-human primate model for human clinical trials, the baboon has similar immune responses, ontogeny, reproductive physiology as a human and develops a human-like schistosomiasis acute syndrome and chronic disease after exposure to schistosome cercariae. The baboon has been used as a 'protection' model to determine the efficacy of schistosome vaccine candidates, including *S. mansoni* 28 glutathione-S-transferase (GST) (Boulanger *et al*. 1991), *S. mansoni* calpain (Sm-p80) (Siddiqui *et al*. 2005; Karmakar *et al*. 2014), *S. mansoni* heat shock protein 70 (HSP70) (Kanamura *et al*. 2002) and attenuated schistosomes including attenuated cercariae (Kariuki *et al*. 2006) and schistosomula (Reid *et al*. 1995). Siddiqui and his colleagues showed recently that the Sm-p80 vaccine generates intricately balanced proinflammatory (Th17 and Th1) responses and, to a much smaller extent, anti-inflammatory (Th2) types of immune responses in the baboon model resulting in both prophylactic and therapeutic efficacies (Karmakar *et al*. 2014).

Based on the current approach for the control of *S. japonicum* in China, studies of protective immunity in bovine schistosomiasis japonica are important when considering the development of a transmission-blocking veterinary vaccine to assist in integrated control efforts (McManus and Dalton, 2006). However, in contrast to murine schistosomiasis, our understanding of the immunology of schistosome infections in water buffaloes and cattle is also very limited. Recently, it has been reported that, following *S. japonicum* infection, worm burdens drop over time in water buffaloes. This self-cure phenomenon appears due to parasite clearance by both immune and non-immune factors, with evidence suggesting that most experimental animals susceptible to schistosomes develop some level of acquired immunity following a primary infection (Li *et al*. 2014*a*). However, studies of PZQ treatment and re-infection in bovines infected with *S. japonicum* in China have suggested that age-related resistance likely occurs in water buffaloes but not in cattle (Wang *et al*. 2006*a*). Furthermore, it was shown that worm length, worm recovery rate and the number of paired worms were significantly increased in yellow cattle, another natural host for *S. japonicum* in China, compared with water buffaloes, in which more serious pathological damage occurs. Immunological analysis suggested that the number of CD4 + T cells, which might constitute an integral component of the immune response, may correlate with worm development in yellow cattle. A shift from Th1- to Th2-type polarized immunity was identified in yellow cattle, but not in water buffaloes infected with schistosomes (Yang *et al*. 2012). Based on the fact that water buffaloes are major reservoirs involved in the transmission of *S. japonicum*, further studies on the immunology of those bovines are necessary to select effective *S. japonicum* transmission vaccine candidates (such as the immune response analysis to migrating larvae, which are the likely targets of an anti-schistosome vaccine) and to determine the desirable route of immunization.

CURRENT STATUS OF VACCINE DEVELOPMENT FOR *S. JAPONICUM*

Highly effective vaccines have been developed against several tapeworm species (Vercruysse *et al*. 2007) indicating it is possible to generate a reliable, high level of protection against complex metazoan parasites, using defined recombinant antigens. A schistosomiasis vaccine is not yet available and substantial development efforts will be required to produce a viable product. Nevertheless, there is evidence indicating that at least partial natural human immunity can develop in schistosomiasis-endemic areas, and that part of this protective effect can be attributed to the immunity that is generated after adult schistosome worms are killed by PZQ. Furthermore, irradiated schistosome cercariae or schistosomula can confer considerable levels of protection against infection in experimental animal challenge models. For example, high levels of protective efficacy (77·62, 88·8 and 99·78% reduction in worm burden, liver eggs and fecal eggs, respectively) against *S. japonicum* challenge were obtained with a ultraviolet-attenuated cercarial vaccine in pigs, with vaccination evoking an effective IFN-γ response and a strong antibody-mediated response, especially in increased levels of IgG2 antibodies (Lin *et al*. 2011*a*). Earlier research in the 1990s showed that water buffaloes vaccinated with irradiation-attenuated *S. japonicum* cercariae gained weight after unattenuated cercarial challenge and developed 89% resistance to *S. japonicum* re-infection (Shi *et al*. 1990). In addition, Chinese bovines (including cattle and buffaloes) immunized with 36 kR γ-irradiated schistosomula reduced the worm burden by 65–76% following a normal cercarial re-challenge (Hsu *et al*. 1984). A major challenge is our limited knowledge of the immunology of schistosome infections in cattle and, especially water buffaloes – due in part to the scarcity of immunological reagents for studying immune responses (McManus and Loukas, 2008). PZQ-treatment and re-infection studies of bovines infected with

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S. japonicum in China have indicated that age-related resistance may occur in water buffaloes but not cattle but whether this self-cure phenomenon has an immunological basis has yet to be determined (Li *et al.* 2014*b*). Additional studies on the immunology of buffaloes and cattle represent an important research area and these will be vital to aid in the process of selecting *S. japonicum* vaccine antigens and in defining optimum routes of immunization. In this respect, a recent study described immunological profiles in previously exposed and re-challenged water buffaloes in China and showed that the intense type-2 immune response at the site of cercarial penetration was significantly different from that seen in naive and permissive animal models, such as mice, suggesting a possible immune mechanism (McWilliam *et al.* 2013). This study also revealed a reduced and delayed immune response in water buffaloes given a high cercarial challenge infection compared with a moderate infection, particularly in the skin and, overall, the study provided new insights of the immunobiology of schistosomiasis in a natural host (McWilliam *et al.* 2013).

Of the current leading *S. japonicum* vaccine candidates, a number include membrane proteins, enzymes and muscle components (Table 1). Detailed information of the characteristics and vaccine efficacy of these and other vaccine candidates can be found elsewhere (McManus and Loukas, 2008). One of the encouraging vaccine targets is paramyosin, a 97-kDa protein (Sj97), which can induce a reduction in worm numbers of 52% in mice and 50% in water buffaloes against *S. japonicum* infection. Sj97 is expressed on the schistosomular tegument and in the acetabular glands. It appears to have similar function as an Fc receptor (Loukas *et al.* 2001) and an exogenous form inhibits activation of the terminal pathway of complement, suggesting a key involvement in host immune evasion (Gobert and McManus, 2005). Currently, Sj97 is in early preclinical process development and in further proof-of-concept studies in mice and water buffaloes (Mo *et al.* 2014). Other important vaccine candidates are *S. japonicum* 26GST (Sj26GST) and *S. japonicum* 28GST (Sj28GST) which have shown encouraging protective efficacy against *S. japonicum* in different mammalian hosts (Table 1). A phase II clinical trial of *S. mansoni* 28GST (Sm28GST) has been carried out (Li *et al.* 2005) and phase I and II trials with *S. haematobium* 28GST (Sh28GST) were completed recently (Mo *et al.* 2014). Three of the leading vaccine candidates against *S. mansoni* – fatty acid-binding protein (Sm14), tetraspanin (Sm-TSP-2) and calpain (Sm-p80) – have been subjected to animal proof-of-concept studies and preclinical process development (Mo *et al.* 2014). Notably, none of these molecules from *S. japonicum* were able to generate effective protection against challenge infection (Liu *et al.* 2004; Wu *et al.* 2005; Zhu, 2005).

Renewed efforts have been made recently to characterize molecules that control schistosome survival, growth and reproduction in order to identify new targets for vaccine development. Accordingly, a series of recently discovered and tested components (including Sj23LHD-GST, Sj-F1, SjCYPB, SjCE-2b, SjTGR, SjAR, SjTPx-2, SjTP22·4, SjEsRRBL1, SjPSMA5, SjB04, SjMF, SjGALE and MLP/HsP70) have been tested as vaccines, details for which are shown in Table 2.

The recent availability of genomic, transcriptomic and proteomic information has allowed the research community to gain new insights into the highly adapted relationship between schistosomes and their mammalian hosts and for identifying novel therapeutic and vaccine targets (Zhou *et al.* 2009; Berriman *et al.* 2009; Young *et al.* 2012). As a result, an insulin receptor was first shown present in *S. japonicum* (Zhou *et al.* 2009). It is striking that schistosomes absorb their dry weight of glucose from their hosts every 5 h (Bueding, 1950), but as they are unable to synthesize insulin (Hu *et al.* 2003), they thus depend on host insulin to facilitate glucose uptake for metabolism, growth and fecundity (You *et al.* 2009). Accordingly, we have isolated 2 types of insulin receptors from *S. japonicum* (SjIRs) that can bind human insulin and which may be involved in modulating the process of glucose uptake in a manner similar to that in *Caenorhabditis elegans* and mammalian cells [56]. Most recently, we showed in a murine vaccine/challenge model that immunization with the L1 subdomain (major insulin-binding domain) of the SjIR (SjLDs) fusion proteins expressed in *Escherichia coli*, conferred highly significant reductions in fecal eggs (56–67%), in liver granuloma density (45–55%) and stunting of adult worms (12–42%), and a reduction in the numbers of mature intestinal eggs (75%) (You *et al.* 2012). Although we did not find a reduction in adult worm burden, our results strongly suggested that the SjLD vaccines were able to induce a significant retardation in the growth of worms and depress fecundity (egg production), emphasizing their potential as encouraging transmission-blocking vaccine candidates. Furthermore, we also found the SjLD vaccines could depress long-term female growth and egg production (unpublished data), thereby inducing long-lived protection against *S. japonicum* infection in the murine model, reinforcing their potential as encouraging transmission-blocking vaccines. Vaccination against schistosomes can be targeted towards the prevention of infection and/or reduced parasite fecundity. A significant reduction in worm burden is regarded as the 'gold standard' for development of anti-schistosome vaccines. However, as schistosome eggs are responsible for pathology and transmission, and the alteration of immune responses in disease progression in schistosomiasis, a vaccine targeting parasite fecundity and/or

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Table 1. Some leading vaccine candidates against *S. japonicum*

Antigen (recombinant protein/DNA)	Abbreviation	Localization	Biological function	Protective efficacy		Reference
				Worm reduction (%)	Egg reduction (%)	
28-kDa GST	Sj28GST	Tegument, parenchyma, oesophageal epithelium and genital organs of male and female worms	Enzyme isoforms that catalyse the detoxification of lipophilic molecules by thio-conjugation	0–69% (mice/ water buffaloes/ sheep)	47.6–72.1%^{F} sheep, 43.1–70.5%^{L} sheep, 50%^{F} buffaloes and 18.6–32.4%^{L} buffaloes	Liu *et al*. (1996) and Shi *et al*. (2001)
26-kDa GST	Sj26GST	Parenchymal region of male worm and in the parenchymal cells between the vitelline glands in the female worm		18.2–30.5% mouse, 61% sheep and 30% cattle	21.7–59%^{L} mouse, 38%^{F} sheep, 50%^{L} water buffalo cattle and 53.5%^{L} pig	Liu *et al*. (1995), Shuxian *et al*. (1997), Taylor *et al*. (1998) and Wu *et al*. (2004)
Paramyosin	Sj97	Schistosomulum surface, tegument and acetabular glands	Binds complement and Fc region of IgG; proposed role in host immune evasion	20–60% mouse, 32.9–34.5% pig and 42–48.8% water buffalo	34–66%^{L} mouse and 57.9%^{I} mouse	Chen *et al*. (2000), McManus (2000) and McManus *et al*. (2001)
Very-low-density lipoprotein-binding protein	SVLBP	Tegument and subtegument of adult male worm	Essential role in lipid acquisition by the parasite and/or in signal transduction pathway	33.4% mouse	47.6%^{L} mouse	Wang *et al*. (2009)
Triose-phosphate isomerase	SjTPI	Surface membranes of newly transformed schistosomulum and most cells of the adult worm	Interferes with invading larva survival	27.9% mouse, 53.6% pig and 41.5% water buffaloes	31.9%^{L} mouse, 49.4%^{L} pig, and 42%^{L} water buffaloes	Zhu *et al*. (2002), Yu *et al*. (2006), Zhu *et al*. (2006) and Da'dara *et al*. (2008)
23-kDa integral membrane protein	Sj23	Surface membrane protein	A member of the tetraspanin protein family which is involved in cell activation, proliferation, motility and metastasis	27–45% mouse, 29–58.6% pig, 59% sheep and 45.5% water buffaloes	33.39–58.4%^{L} mouse, 48.2–56.4%^{L} pig, 35–58%^{F} sheep, 56–66%^{L} sheep and 42.9%^{L} water buffaloes	Taylor *et al*. (1998), Zhu *et al*. (2004), Gan *et al*. (2004, 2005), Wu *et al*. (2005) and Da'dara *et al*. (2008)
Calpain Calcium-activated neutral proteinase	SjP80	Penetration glands and in the secretions of cercariae	An important antigenic protein involved in surface membrane renewal/recycling	41.2% mouse		Zhang *et al*. (2001) and Ohta *et al*. (2004)
Fatty acid-binding protein	SjFABP (Sj14)	Within lipid droplets below the subtegumetal region of the male parasite and in the vitelline droplets of the vitelline glands of female worms	Important for uptake, transport and compartmentalization of host-derived fatty acids, playing a vital role in the physiology and survival of the parasite	24.1–49% mouse, 31.9% rat and 59.2% sheep	20–27.2%^{L} mouse, 23.6–63.3%^{F} sheep, 44.9%^{L} sheep and 69.6%^{I} sheep	Gobert *et al*. (1997), Liu *et al*. (2004), Wu *et al*. (2005) and Zhu (2005)

L, liver egg reduction; F, fecal egg reduction; I, intestine egg reduction.

Table 2. Recently tested vaccine candidates against *S. japonicum* infection

Antigen	Abbreviation	Biological function or immunolocalization/other properties	Protective efficacy, reduction (%)		Reference
			Worm	Egg	
Thioredoxin glutathione reductase	SjTGR	Important for worm development and survival	27.8–38.8		Han *et al*. (2012) and Cao *et al*. (2013)
Aldose reductase	SjAR	Enzyme involved in the antioxidant defence system; distributed in the gynecophoral canal of adult male worms	32.90	42.8[L]	Liu *et al*. (2013)
Thioredoxin peroxidize-2	SjTPx-2	Antioxidative enzyme acting as a scavenger against reactive oxygen species (ROS) generated outside of schistosome worms to prevent the oxidation of the bodies and/or attack by immune cells producing the ROS	31.20		Hong *et al*. (2013)
Thyroid hormone receptor beta	SjTHRβ	Interacts with thyroid hormones and important in modulating growth, development and differentiation, and metabolic processes	27.50		Qiu *et al*. (2012)
Sj-F1	Sj-F1	Discovered by screening an adult worm cDNA library with female antigen-immunized sera. Function of expressed protein unknown	21.5	34.8[L]	Wang *et al*. (2013)
Cyclophilin B	SjCyPB	A prolyl isomerize with highest expression in 18-day schistosomula	31.50	41[L]	Peng *et al*. (2010)
Calcium-binding tegumental protein	SjTP22.4	On surface of tegument of adult and schistosomulum and cercaria and in the parenchymatous tissues and intestinal epithelium		32[L] 41[M]	Zhang *et al*. (2012*d*)
Estrogen-related receptor beta like 1	SjEsRRBL1	A sex hormone receptor, highly expressed in schistosomula	31	35[L]	Wu *et al*. (2012)
Nucleotide pyrophosphatase/ phosphodiesterase-5		Important in the hydrolysis of pyrophosphate or phosphodiester bonds in nucleotides and their derivatives, and located surface of the male adult worms	29.90		Zhang *et al*. (2012*a*)
Oncomelania hupensis haemocyanin	OhH	A giant oxygen transport protein isolated from *O. hupensis*, which shares carbohydrate epitopes with different developmental stages of *S. japonicum* (cercaria, schistosomulum, adult worm and egg) and exhibited serological cross-reactivity with these stages of *S. japonicum* immune sera	52.50	69.2[L]	Guo *et al*. (2011)
Proteasome subunit α type 5	SjPSMA5	A member of the peptidase T1A family that is a 20S core α subunit. Up-regulated in 18- and 32-day-old schistosomes, with the level of expression in males around 4-fold higher than that in female worms at 42 days	23.30	35.2[L]	Hong *et al*. (2010)
Metalloprotease gene	SjB04	Located in the intestinal epithelium of adult worms	27.10	57.8[F]	Xu *et al*. (2011*a*)
Myoferlin	SjMF	Involved in plasma membrane repair Highly expressed in 42-day-old worms, significantly higher in female worms. Distribution on the surface of worms at different stages	21.8–23.21	42.6–28.4[L]	Xiong *et al*. (2013)
Uridine diphosphate-glucose 4-epimerase protein	SjGALE	Located on the tegument of worms	34	49[L]	Liu *et al*. (2012)
Mortalin-like protein	MLP/Hsp70	A member of the HSP70 family and plays a key regulatory role in parasite development and pathogenesis. It has potential as an adjuvant	31.31	58.59[I]	He *et al*. (2010)

L, liver egg reduction; F, fecal egg reduction; I, intestine egg reduction; M, egg mature rate.

egg viability may represent a more realistic strategy for vaccine development (McManus and Loukas, 2008). In order to obtain optimum vaccine efficacy, one logical approach is to design multivalent vaccines targeting 2 or more antigens which promote the depression of adult parasite growth and egg production and a reduction in worm numbers.

Another lead vaccine candidate against *S. japonicum* infection that is involved in glucose metabolism is triose-phosphate isomerase (SjCTPI), which reduces worm burdens in mice (27·9%) (Zhu *et al*. 2002), pigs (48%) (Zhu *et al*. 2006) and water buffaloes (48–52%) (Yu *et al*. 2006; Da'dara *et al*. 2008). This enzyme is able to convert glyceraldehyde-3-phosphate to dehydroxyacetone phosphate, which is a key step in the glycolytic pathway, whereby a cell breaks down glucose into energy. TPI is located in most cells of schistosome worms and on the surface membranes of newly transformed schistosomula, the stage most likely to be the target of an anti-schistosome vaccine. Vaccination with a SjCTPI DNA vaccine had a significant effect in reducing female worm burdens (53·6–59·6%) and liver egg numbers (49·4–65·8%) in the pig model of schistosomiasis japonica; in addition, the granuloma sizes in vaccinated animals were reduced by 42% (Zhu *et al*. 2012). The efficacy of SjCTPI and the tetraspanin, SjC23, on their own, and as fusions with the heat-shock protein 70 (Hsp70) were assessed as DNA vaccines in water buffaloes in China against *S. japonicum* challenge (Da'dara *et al*. 2008). The most encouraging vaccine was the SjCTPI-Hsp70 construct, that generated 51·2, 61·5 and 52·1% reduction in worm burden, hepatic eggs and fecal eggs, respectively (Da'dara *et al*. 2008). The SjCTPI-Hsp70 vaccine is currently undergoing field testing in bovines in China and the Philippines.

It is now clear that several *S. japonicum* vaccine candidates are able to induce levels of between 50 and 70% protective efficacy in vaccination/challenge experiments with mice and larger mammalian species, with further immunization boosts increasing these levels so that their further development for veterinary use is a realistic proposition (McManus and Loukas, 2008). However, further study is necessary on the development of multivalent vaccines and novel adjuvants to improve on these levels of protection (Siddiqui *et al*. 2011; You *et al*. 2014). Furthermore, there are some challenges that will need to be overcome, including the possible risk of atopic IgE responses generated by the various vaccines, the use of appropriate adjuvant/vaccine formulations, whether vaccine efficacy is reduced due to co-infection with other pathogens in schistosomiasis-endemic areas, and the practical requirement of developing a vaccine that can be given as a single dose, ideally orally, without the requirement of boosting.

DEVELOPMENT AND TESTING OF NEW DIAGNOSTICS FOR SCHISTOSOMIASIS

To date, selective chemotherapy with PZQ is one of the components of schistosomiasis control programmes so that correct diagnosis of infected individuals is important. However, a sensitive and specific assay for field diagnosis of schistosomiasis japonica that is simple and affordable is not currently available. This poses a barrier to control efforts leading to schistosomiasis elimination, especially when the schistosome prevalence drops to low levels, as is now occurring in China [41]. Hence, the search for novel diagnostic tools for schistosomiasis is recognized as an urgent priority. Generally, *S. japonicum* infections can be detected by 3 approaches: parasitological, immunological and molecular.

Parasitological methods

Detection of eggs in stool samples is the customary method for diagnosing schistosome infections. The Kato-Katz (KK) thick smear technique is the most widely used procedure, being the standard method recommended by the WHO for both qualitative and quantitative diagnoses of intestinal schistosomiasis. However, the sensitivity of the KK method can vary from 40 to 100%, with the negative predictive values ranging from 52·5 to 100% (Zhou *et al*. 2007). Furthermore, if only a single KK slide is prepared from a single stool specimen, sensitivity is low (Lin *et al*. 2011*b*). This leads to a marked underestimation of the prevalence and intensity of infection, particularly in low prevalence areas (Lier *et al*. 2008), and this can confound confirmation of cure after PZQ treatment (Berhe *et al*. 2004). Ideally, multiple fecal examinations (typically 2 fecal samples per individual; 3 KK slides each sample) should be undertaken to reduce the false-negative results, but this is time and labour intensive and compliance drops with the number of stool samples requested. If an endemic population is first screened serologically by indirect haemagglutination assay (IHA) or enzyme-linked immunosorbent assay (ELISA) and seropositives confirmed by the KK method, correlation analysis indicates that the positivity rate with KK rises with the number of fecal specimens and slides used. Those individuals that were egg-positive but negative by IHA or ELISA were mainly cases with low infection intensity (Zhang *et al*. 2012*a*).

It is important to obtain purified eggs free from fecal components in order to increase diagnostic sensitivity and improve microscopic visualization of *S. japonicum* eggs. Two novel egg purification methods were recently described, which have potential for use in areas with low infection intensity, or where there is suspected elimination of schistosomiasis japonica following control efforts. The formalin-ethyl acetate sedimentation-digestion

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(FEA-SD) technique (Xu *et al*. 2012) is effective for identifying and quantifying *S. japonicum* eggs in fecal samples from infected bovines in endemic areas. FEA-SD removes about 70% of debris from fecal samples and the remaining material is translucent. Another method for *Schistosoma* egg detection has been developed that is based on magnetic fractionation of parasite eggs from fecal matter (Fagundes Teixeira *et al*. 2007). With this approach, termed Helmintex, magnetic microspheres are mixed with fecal samples to make parasite egg–magnetic microsphere conjugates. The magnetic microspheres and eggs are then co-purified from other fecal material using a magnetic field and field gradient. The concentrated and purified eggs are more easily detectable by microscopy. This method is able to screen larger sample volumes, resulting in improved diagnostic sensitivity, although the mechanism of formation of the conjugates is unknown but may reflect the specific surface features of eggs and microspheres, or to their magnetic properties (Karl *et al*. 2013).

Immunological methods

Patent schistosome infections are generally highly immunogenic, and anti-schistosome antibodies can be readily detected in subjects using a wide range of immunodiagnostic techniques. Many variations of indirect immunological approaches in schistosomiasis diagnosis include the circumoval precipitin test (COPT) and the IHA which have been widely used historically, and ELISA and dipstick dye immunoassays (DDIA), which have been used more frequently in the last 20 years. ELISA, using soluble egg antigen (SEA) as the source of target antigen, is the most widely used technique currently (Doenhoff *et al*. 2004). HSP70 and 78 kDa glucose-regulated protein, both present in SEA, may have diagnostic value for detecting early *S. japonicum* infections (Wang *et al*. 2012). The modified fast ELISA (F-ELISA), which combines the 2-step routine ELISA into a single step making the assay process faster and simpler without sacrificing diagnostic accuracy (Hua *et al*. 2011), may provide a rapid and practical method for schistosomiasis diagnosis in the field. Recently, more rapid, sensitive and portable diagnostic assays to detect human antibodies against *S. japonicum*, have been developed and tested in areas of low endemicity for schistosomiasis japonica in China (Table 3). These methods include: (1) DDIA (Xu *et al*. 2011*a*) which is commercially available in China, and the dipstick with latex immunochromatographic assay (DLIA) (Yu *et al*. 2011); (2) magnetic affinity enzyme-linked immunoassays (MEIAs), based on the signal transduction protein 14-3-3 (Sj14-3-3), recombinant 26 kDa GST (rSj26GST) (Yu *et al*. 2012*a*), or SEA-MEIA (Yu *et al*. 2012*b*); (3) a time-resolved fluoroimmunoassay (TRFIA), established for detecting Sj14-3-3, as a circulating antigen in serum (Qian *et al*. 2011); (4) an electrochemical immunosensor array (ECISA) assay (Deng *et al*. 2013) which uses a recombinant *S. japonicum* calcium-binding protein (SjE16), with SEA, co-immobilized on a disposable 16-channel screen-printed carbon electrode array. Antibodies in serum samples can be detected by a portable electrochemical detector; (5) a multiplexed bioanalytical assay which is developed by fusing 2 types of gold nanorods (GNRs) (Huang *et al*. 2012). This technique allows the serum-based diagnosis of subjects infected with *S. japonicum* without the need for sample pretreatment and it can diagnose individuals co-infected with schistosomiasis and TB.

Serodiagnosis of schistosomiasis suffers from a number of drawbacks common to the antibody-based detection of other parasitic infections (Doenhoff *et al*. 2004; Rabello and Enk, 2006). One difficulty is in identifying an active from a previous infection, as parasite-specific antibodies remain detectable long after cure, and another is the inability to quantify infection intensity. A range of approaches to improve the accuracy of immunodiagnostic assays have been described. These include using specific parasite extracts such as cercarial antigens (Chand *et al*. 2010), using recombinant proteins as immunodiagnostic targets (Zhou *et al*. 2010) or using a pool of synthetic peptides selected on the basis of the amino acid sequence of proteins from different antigenic schistosome preparations (de Oliveira *et al*. 2008). Another approach that has been used with success in China is to combine information from serological surveys with parasitological data and to use a Bayesian statistical approach to develop accurate epidemiological maps of infection prevalence (Wang *et al*. 2006*b*). So far, a number of candidate antigens have been tested for diagnosing *S. japonicum* infection; these include Sj23HD (Wang *et al*. 2011*b*), rSj26GST-Sj32 (Cai *et al*. 2011), SjEFCAB (Lu *et al*. 2012*b*), SjTPx-1 (Angeles *et al*. 2012) and Sj1TR (Angeles *et al*. 2012), Sj7TR (Angeles *et al*. 2011), SjCHGCS19 (Guo *et al*. 2012), SjLAP (Zhang *et al*. 2011), whose characteristics are shown in Table 4. Recently, Xu *et al*. (2014) undertook a genome-wide survey to discover diagnostic protein markers for *S. japonicum* infection. In the study, 204 *S. japonicum* predicted secreted proteins (SjSPs) were arrayed on glutathione-immobilized microplates and screened with 302 patient serum samples that were diagnosed by the KK method as egg positive. One protein, SjSps-13, was identified as a potential diagnostic protein marker with 90·4% sensitivity and 98·9% specificity and its diagnostic validity was tested in ELISA using 1371 resident samples in a field study. The current scarcity of effective diagnostic tools is a dominant factor precluding the effective management of schistosomiasis (Colley *et al*. 2014) so

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Table 3. Immunoassays for diagnosing *S. japonicum* infection

Diagnostic assay	Description	Cross–reactivity	Reference
DDIA	DDIA was compared with stool examination to evaluate its accuracy as a primary approach for screening the human population in 7 villages of low endemicity; the DDIA achieved 91.3% sensitivity and 99.3% specificity. It is commercially available in China	Not evaluated	Xu *et al*. (2011*b*)
DLIA	Using latex microspheres as a colour probe, DLIA showed 95.1% sensitivity and 94.9% specificity in comparison with a classical ELISA	No cross-reactivity with Clonorchiasis, intestinal nematodes, or *Angiostrongylus cantonensis*, but 42% cross-reactivity with paragonimiasis	Yu *et al*. (2011)
Immunomagnetic bead ELISA based on IgY (egg yolk immunoglobulin)	Detection of circulating antigens in sera of hosts infected with *S. japonicum;* 100% sensitivity with acute cases and 91.5% sensitivity with chronic cases was achieved	3.3% cross-reactivity with clonorchiasis but no cross-reactivity with paragonimiasis	Lei *et al*. (2011, 2012)
MEIA	Based on Sj14-3-3 With a higher ratio of the mean positive value to the mean negative value (P/N) at the same dilution ratio in infected mice compared with ELISA Based on rSj26GST Based on SEA N/P	No cross reactivity	Yu *et al*. (2012*a*, *b*) and Yu *et al*. (2014)
TRFIA	Detection of the *S. japonicum* signal transduction protein 14-3-3 as a circulating antigen in serum	Not evaluated	Qian *et al*. (2011)
ECISA	Recombinant SjE16 is used as a principal antigen, while the SEA acts as a minor, co-assembling agent, which are co-immobilized on a disposable 16-channel screen-printed carbon electrode array	100% sensitivity with high specificity	Deng *et al*. (2013)
GNRs	A multiplexed bioanalytical assay with 100% sensitivity	Not evaluated	Huang *et al*. (2012)

Table 4. Novel diagnostic molecules to detect early *S. japonicum* infection

Schistosome antigen	Description	Sensitivity (%)	Specificity (%)	Cross-reactivity	Reference
Sj23HD	Hydrophilic domain (HD) of the 23-kDa (tetraspanin) membrane protein with y diagnostic value for early detection of schistosome infection in mice	Higher than SEA-ELISA	Higher than SEA-ELISA	No evaluated	Wang *et al*. (2011*b*)
rSj26GST-Sj32	rSj26-Sj32-IgG-ELISA applicable for the immunodiagnosis for chronic schistosomiasis japonica	95.00%^{H} (38/40)	97.67%^{H} (42/43)	No cross-reactivity in ELISA with sera from patients with alveolar echinococcosis	Cai *et al*. (2011)
SjEFCAB	*Schistosoma japonicum* calcium-binding EF-hand domain containing protein	82.1%^{H} (64/78)	95.0%^{H} (76/80)	The cross-reactivities with sera of *Clonorchiasis sinensis*, Cysticercosis and trichinosis patients were 1/5, 1/10 and 1/9, respectively. There was no cross-reaction with sera of *Paragonimiasis westermani* patients	Lu *et al*. (2012*b*)
SjTPx-1	Thioredoxin peroxidase-1 protein	82.61%^{W} 85.71%^{H}	78.26%^{W}	No or very minimal cross-reactivity with other parasitic infections	Angeles *et al*. (2011, *2012*)
Sj1TR	Tandem repeat protein	82.61%^{W}	78.26%^{W}		
Sj7TR		85.71%^{H}			
SjCHGCS19	Novel 303-bp DNA sequence from non-long terminal repeat retrotransposon, amplified from the sera of rabbits l at 3 dpi by nested-PCR which became negative at 17 weeks post-treatment.	97.67%^{H}	96.07%^{H}	No cross-reactions were detected in DNA samples representing *C. sinensis* and *Trichinella spirals*	Guo *et al*. (2012)
SjSP-13	*Schistosoma japonicum* putative secreted protein, identified by arraying SjSPs on glutathione-immobilized microplates and screening with positive infected human serum samples.	90.4%	98.9%	2% cross-reactivity with clonorchiasis in 51 serum samples	Xu *et al*. (2014)

W, water buffaloes; H, human; SEA, soluble egg antigen.

the application of this newly developed sensitive, specific and affordable rSP13-ELISA method should help reduce schistosomiasis transmission through targeted treatment of individuals, particularly with low-intensity infections, and therefore support schistosomiasis control and elimination strategies (Xu *et al*. 2014).

An early study using immunoassays demonstrated the presence of schistosome-derived antigens in the circulation and/or excreta of hosts with schistosomiasis (Deelder *et al*. 1976), and this report stimulated considerable research on antigen detection as a means of diagnosing the disease. Indeed, detection of schistosome circulating antigens (CAs) has now been shown to be an efficient method to differentiate between previous exposure and current infection. In one approach, AWA egg yolk immunoglobulin (IgY) was generated by immunization of hyline chicken hens with AWA. The purified IgY was then immobilized onto resin to immune-precipitate CAs in sera from infected subjects. Four proteins including protein BUD31 homologue, ribonuclease, SJCHGC06971 protein and SJCHGC04754 protein were isolated from the precipitated proteins that could be employed as diagnostic biomarkers (Lu *et al*. 2012*a*). A novel immunomagnetic bead ELISA, based on IgY, has also been used for detection of circulating CAs in sera or urine of hosts infected with *S. japonicum* (Lei *et al*. 2011, *2012*) (Table 3).

Molecular methods

Some of the deficiencies of currently available diagnostic methods for intestinal schistosomiasis are that: (1) early diagnosis of the disease using egg detection methods is problematical. Generally, egg production within the host takes several weeks as does the passage of eggs through the gut wall, their discharge into the intestinal lumen and their release in feces; (2) variability in egg shedding and problems in distribution of eggs in the analysed sample often leads to unreliable results when microscopic examination for eggs is performed; (3) as emphasized earlier, serological tests, based on antibody detection, do not discriminate between active infection and previous exposure. Accordingly, the application of the polymerase chain reaction (PCR) as a tool for the diagnosis of schistosomiasis has been explored for detection of schistosome DNA in human and bovines feces (Fung *et al*. 2012), in serum/plasma (Wichmann *et al*. 2009) and in urine (Obeng *et al*. 2008) and the approach has proven to be of value (Xu *et al*. 2013). Some of these highly sensitive and specific PCR-based methods have been assessed in areas with medium or low intensity of schistosome infection. A combination of real-time PCR (qPCR) and the earlier described FEA-SD technique (Xu *et al*. 2012) was used in the Philippines to determine the prevalence and intensity of *S. japonicum*, thereby providing a more accurate diagnosis to evaluate the potential role of bovines in the transmission of *S. japonicum* (Gordon *et al*. 2012). It should be stressed, however, that PCR-based methods give positive results only if the analysed fecal sample contains schistosome DNA and inhibitors present in feces may affect the PCR assay working optimally.

In another diagnostic approach, Wichmann *et al*. (2009) used real-time PCR to detect cell free parasite DNA (CFPD) in human plasma according to the principle that *Schistosoma* worms contain DNA copies, which may be released due to parasite turnover and reach the blood, in stoichiometrical excess over parasite count and that schistosome DNA. This method may provide a new laboratory tool to detect schistosome infection in all phases of clinical disease (Wichmann *et al*. 2009).

Another molecular approach is loop-mediated isothermal amplification (LAMP), a highly sensitive DNA detection method that is proving of value for the diagnosis of a number of pathogenic organisms including schistosomes. A LAMP assay has been established that detects *S. japonicum* DNA in fecal and serum samples of rabbits and in sera of infected human subjects. Based on the sequence of a highly repetitive retrotransposon, SjR2, the LAMP method was considerably more sensitive than traditional PCR being able to measure 0·08 fg *S. japonicum* and appropriate for clinical diagnosis and therapeutic evaluation of human schistosomiasis (Xu *et al*. 2010). LAMP appears suitable for the detection of early *S. japonicum* infection in certain patients including travellers, migrants, military personnel and younger aged subjects but it is likely to be of less value for determining the efficacy of drug treatment in the early stages because of its high sensitivity (Wang *et al*. 2011*a*).

Recently, circulating microRNAs (miRNAs) have attracted attention as novel biomarkers for the diagnosis of schistosomiasis and also for further understanding the host–schistosome interaction. Deep sequencing identified 5 schistosome-specific miRNAs (Bantam, miR-3479, miR-10, miR-3096 and miR-0001) in the plasma of *S. japonicum*-infected rabbits, 4 of which were detectable by real-time reverse transcription-PCR in the plasma of mice infected with *S. japonicum* (Cheng *et al*. 2013). Another miRNA molecule, miR-223, was identified by He *et al*. (2013) as a potential new biomarker of *S. japonicum* infection and the assessment of the response to PZQ treatment. Parasite-derived miRNAs have also been identified (Anna *et al*. 2014) as novel markers of *S. mansoni* infection in mice and humans, some of which could distinguish 'egg-negative' from 'egg-positive' individuals with high specificity and sensitivity with potential diagnostic value. However, this study showed that, whereas several host miRNAs were shown to be dysregulated in the livers of mice

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during *S. mansoni* infection, they were unable to serve as reliable serum biomarkers of infection in humans (Anna *et al*. 2014). These data contrast with those of Han *et al*. (2013) who applied a miRNA microarray to investigate differences in miRNA expression in different tissues, including the liver, of mice before and 10 dpi with *S. japonicum* and detected a total of 220 miRNAs in the different tissues whose functions correlated with nutrient metabolism, the immune response, apoptosis, signalling pathways and cell differentiation (Han *et al*. 2013). As pointed out by Hoy *et al*. (2014), these conflicting results may have been due to the very early time point (10 dpi) used in the *S. japonicum* study.

FUTURE DIRECTIONS

The key to eliminating schistosomiasis is to reduce transmission combined with morbidity control, an approach that is more cost effective than treating the clinical outcome of continued re-infection, and which is currently being used successfully in China (Sun *et al*. 2011). Central to this goal is to integrate traditional control measures – PZQ treatment, the use of molluscicides, environmental modification, health education/promotion, enhanced water supply (Kosinski *et al*. 2012) and improved sanitation – with an effective vaccine, a tool that is needed to accelerate intervention efforts to eliminate a disease that has existed for many centuries. In this respect, the Chinese experience serves as a good model, whereby a comprehensive control approach based on interventions to block transmission of *S. japonicum* infection from bovines and humans to snails has proven highly effective (Seto *et al*. 2011). As emphasized earlier, it has been established that bovines are the major animal reservoir host for *S. japonicum* in China and the Philippines (McManus and Loukas, 2008) and this fact underpins efforts to develop a bovine transmission-blocking vaccine against *S. japonicum* as an effective and feasible objective. Recent developments in the preclinical and clinical testing of existing anti-schistosome vaccine candidates have been encouraging (Mo *et al*. 2014). Furthermore, the most recent comprehensive understanding of schistosome genomes and proteomes has equipped us with all the information for antigen choice as novel vaccine targets. Based on the fact that the membrane proteins located on the tegument of the schistosomulum and the adult worm are rational vaccine targets (Loukas *et al*. 2007), we need to select the best antigen or combined antigens as a schistosomiasis vaccine using other innovative tools. Defining a clear target product profile (TPP) for an optimum schistosomiasis vaccine and using new technologies and tools which are available for new antigen discovery, clinical research and vaccine efficacy assessment will all contribute to accelerated progress (Mo *et al*. 2014). Effective schistosomiasis vaccines and new drugs that act on both adult and larval schistosomes would help to achieve and sustain disease control and eventual elimination. Importantly as well, further research, similar to that described by Xu *et al*. (2014), is required to develop improved diagnostic tests that are able to identify light *S. japonicum* infections so as to determine the extent of the interruption of transmission in an endemic area and to establish whether control efforts have led to schistosomiasis elimination.

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REFERENCES

Angeles, J. M., Goto, Y., Kirinoki, M., Leonardo, L., Tongol-Rivera, P., Villacorte, E., Inoue, N., Chigusa, Y. and Kawazu, S. (2011). Human antibody response to thioredoxin peroxidase-1 and tandem repeat proteins as immunodiagnostic antigen candidates for *Schistosoma japonicum* infection. *American Journal of Tropical Medicine and Hygiene* **85**, 674–679.

Angeles, J. M., Goto, Y., Kirinoki, M., Asada, M., Leonardo, L. R., Rivera, P. T., Villacorte, E. A., Inoue, N., Chigusa, Y. and Kawazu, S. (2012). Utilization of ELISA using thioredoxin peroxidase-1 and tandem repeat proteins for diagnosis of *Schistosoma japonicum* infection among water buffaloes. *PLoS Neglected Tropical Diseases* **6**, e1800.

Hoy, A. M., Lundie, R. J., Ivens, A., Quintana, J. F., Nausch, N., Forster, T., Jones, F., Kabatereine, N. B., Dunne, D. W., Mutapi, F., MacDonald, A. S. and Buck, A. H. (2014). Parasite-derived microRNAs in host serum as novel biomarkers of Helminth infection. *PLoS Neglected Tropical Diseases* **8**, e2701.

Berhe, N., Medhin, G., Erko, B., Smith, T., Gedamu, S., Bereded, D., Moore, R., Habte, E., Redda, A., Gebre-Michael, T. and Gundersen, S. G. (2004). Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. *Acta Tropica* **92**, 205–212.

Berriman, M., Haas, B. J., LoVerde, P. T., Wilson, R. A., Dillon, G. P., Cerqueira, G. C., Mashiyama, S. T., Al-Lazikani, B., Andrade, L. F., Ashton, P. D., Aslett, M. A., Bartholomeu, D. C., Blandin, G., Caffrey, C. R., Coghlan, A., Coulson, R., Day, T. A., Delcher, A., DeMarco, R., Djikeng, A., Eyre, T., Gamble, J. A., Ghedin, E., Gu, Y., Hertz-Fowler, C., Hirai, H., Hirai, Y., Houston, R., Ivens, A., Johnston, D. A., *et al.* (2009). The genome of the blood fluke *Schistosoma mansoni*. *Nature* **460**, 352–358.

Boulanger, D., Reid, G. D., Sturrock, R. F., Wolowczuk, I., Balloul, J. M., Grezel, D., Pierce, R. J., Otieno, M. F., Guerret, S., Grimaud, J. A., Butterworth, A. E. and Capron, A. (1991). Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. *Parasite Immunology* **13**, 473–490.

Bueding, E. (1950). Carbohydrate metabolism of *schistosoma mansoni*. *Journal of General Physiology* **33**, 475–495.

Cai, S. F., Li, W. G. and Wang, M. (2011). Diagnosis of chronic *schistosomiasis japonicum* with the recombinant Sj26GST-Sj32 fusion protein by ELISA. *Sichuan Da Xue Xue Bao. Yi Xue Ban (Journal of Sichuan University. Medical Science Edition)* **42**, 331–334.

Cao, Y., Zhao, B., Han, Y., Zhang, J., Li, X., Qiu, C., Wu, X., Hong, Y., Ai, D., Lin, J. and Fu, Z. (2013). Gene gun bombardment with DNA-coated golden particles enhanced the protective effect of a DNA vaccine

https://doi.org/10.1017/S0031182014001310 Published online by Cambridge University Press

based on thioredoxin glutathione reductase of *Schistosoma japonicum*. *Biomed Research International* **2013**, 952416.

Chand, M. A., Chiodini, P. L. and Doenhoff, M. J. (2010). Development of a new assay for the diagnosis of schistosomiasis, using cercarial antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 255–258.

Chen, D., Luo, X., Xie, H., Gao, Z., Fang, H. and Huang, J. (2013*a*). Characteristics of IL-17 induction by *Schistosoma japonicum* infection in C57BL/6 mouse liver. *Immunology* **139**, 523–532.

Chen, H., Nara, T., Zeng, X., Satoh, M., Wu, G., Jiang, W., Yi, F., Kojima, S., Zhang, S. and Hirayama, K. (2000). Vaccination of domestic pig with recombinant paramyosin against *Schistosoma japonicum* in China. *Vaccine* **18**, 2142–2146.

Chen, D., Xie, H., Luo, X., Yu, X., Fu, X., Gu, H., Wu, C., Tang, X. and Huang, J. (2013*b*). Roles of Th17 cells in pulmonary granulomas induced by *Schistosoma japonicum* in C57BL/6 mice. *Cellular Immunology* **285**, 149–157.

Chen, H. and Lin, D. (2004). The prevalence and control of schistosomiasis in Poyang Lake region, China. *Parasitology International* **53**, 115–125.

Cheng, G., Luo, R., Hu, C., Cao, J. and Jin, Y. (2013). Deep sequencing-based identification of pathogen-specific microRNAs in the plasma of rabbits infected with *Schistosoma japonicum*. *Parasitology* **140**, 1751–1761.

Colley, D. G., Bustinduy, A. L., Secor, W. E. and King, C. H. (2014). Human schistosomiasis. *The Lancet* **383**, 2253–2264.

Da'dara, A. A., Li, Y. S., Xiong, T., Zhou, J., Williams, G. M., McManus, D. P., Feng, Z., Yu, X. L., Gray, D. J. and Harn, D. A. (2008). DNA-based vaccines protect against zoonotic schistosomiasis in water buffalo. *Vaccine* **26**, 3617–3625.

Deelder, A. M., Klappe, H. T., van den Aardweg, G. J. and van Meerbeke, E. H. (1976). *Schistosoma mansoni*: demonstration of two circulating antigens in infected hamsters. *Experimental Parasitology*, **40**, 189–197.

de Jong, E. C., Vieira, P. L., Kalinski, P., Schuitemaker, J. H., Tanaka, Y., Wierenga, E. A., Yazdanbakhsh, M. and Kapsenberg, M. L. (2002). Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse Th cell-polarizing signals. *Journal of Immunology* **168**, 1704–1709.

Deng, W., Xu, B., Hu, H., Li, J., Hu, W., Song, S., Feng, Z. and Fan, C. (2013). Diagnosis of schistosomiasis japonica with interfacial co-assembly-based multi-channel electrochemical immunosensor arrays. *Scientific Reports* **3**, 1789.

de Oliveira, E. J., Kanamura, H. Y., Takei, K., Hirata, R. D., Valli, L. C., Nguyen, N. Y., de Carvalho Rodrigues, I., de Jesus, A. R. and Hirata, M. H. (2008). Synthetic peptides as an antigenic base in an ELISA for laboratory diagnosis of *Schistosomiasis mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 360–366.

de Oliveira Fraga, L. A., Lamb, E. W., Moreno, E. C., Chatterjee, M., Dvorak, J., Delcroix, M., Sajid, M., Caffrey, C. R. and Davies, S. J. (2010). Rapid induction of IgE responses to a worm cysteine protease during murine pre-patent schistosome infection. *BMC Immunology* **11**, 56.

Diallo, T. O., Remoue, F., Schacht, A. M., Charrier, N., Dompnier, J. P., Pillet, S., Garraud, O., N'Diaye A, A., Capron, A., Capron, M. and Riveau, G. (2004). Schistosomiasis co-infection in humans influences inflammatory markers in uncomplicated *Plasmodium falciparum* malaria. *Parasite Immunology* **26**, 365–369.

Doenhoff, M. J., Chiodini, P. L. and Hamilton, J. V. (2004). Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? *Trends in Parasitology*, **20**, 35–39.

Du, X., Wu, J., Zhang, M., Gao, Y., Zhang, D., Hou, M., Ji, M. and Wu, G. (2011). Upregulated expression of cytotoxicity-related genes in IFN-gamma knockout mice with *Schistosoma japonicum* infection. *Journal of Biomedicine and Biotechnology* **2011**, 864945.

Elias, D., Akuffo, H., Pawlowski, A., Haile, M., Schon, T. and Britton, S. (2005). *Schistosoma mansoni* infection reduces the protective efficacy of BCG vaccination against virulent *Mycobacterium tuberculosis*. *Vaccine* **23**, 1326–1334.

El Ridi, R., Tallima, H., Mahana, N. and Dalton, J. P. (2010). Innate immunogenicity and in vitro protective potential of *Schistosoma mansoni* lung schistosomula excretory–secretory candidate vaccine antigens. *Microbes and Infection* **12**, 700–709.

Fagundes Teixeira, C., Neuhauss, E., Ben, R., Romanzini, J. and Graeff-Teixeira, C. (2007). Detection of *Schistosoma mansoni* eggs in feces through their interaction with paramagnetic beads in a magnetic field. *PLoS Neglected Tropical Diseases* **1**, e73.

Fallon, P. G., Richardson, E. J., McKenzie, G. J. and McKenzie, A. N. (2000). Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *Journal of Immunology* **164**, 2585–2591.

Fung, M. S., Xiao, N., Wang, S. and Carlton, E. J. (2012). Field evaluation of a PCR test for *Schistosoma japonicum* egg detection in low-prevalence regions of China. *American Journal of Tropical Medicine and Hygiene* **87**, 1053–1058.

Gan, Y., Shi, Y. E., Bu, L. Y., Ning, C. X. and Zhu, H. G. (2005). Vaccination of mice with recombinant nucleic acid vaccine encoding the integral membrane protein Sj23 and cytokine IL-12 elicits specific immune responses against *Schistosoma japonica*. *Zhonghua Yi Xue Za Zhi* **85**, 193–198.

Gan, Y., Shi, Y. E., Bu, L. Y., Zhu, X. H., Ning, C. X. and Zhu, H. G. (2004). Immune responses against *Schistosoma japonicum* after vaccinating mice with a multivalent DNA vaccine encoding integrated membrane protein Sj23 and cytokine interleukin-12. *Chinese Medical Journal* **117**, 1842–1846.

Gobert, G. N. and McManus, D. P. (2005). Update on paramyosin in parasitic worms. *Parasitology International* **54**, 101–107.

Gobert, G. N., Stenzel, D. J., Jones, M. K. and McManus, D. P. (1997). Immunolocalization of the fatty acid-binding protein Sj-FABPc within adult *Schistosoma japonicum*. *Parasitology* **115** (Pt 1), 33–39.

Gonnert, R. and Andrews, P. (1977). Praziquantel, a new board-spectrum antischistosomal agent. *Zeitschrift fur Parasitenkunde* **52**, 129–150.

Gordon, C. A., Acosta, L. P., Gray, D. J., Olveda, R. M., Jarilla, B., Gobert, G. N., Ross, A. G. and McManus, D. P. (2012). High prevalence of *Schistosoma japonicum* infection in Carabao from Samar Province, the Philippines: implications for transmission and control. *PLoS Neglected Tropical Diseases* **6**, e1778.

Guo, J., Li, Y., Gray, D., Ning, A., Hu, G., Chen, H., Davis, G. M., Sleigh, A. C., Feng, Z., McManus, D. P. and Williams, G. M. (2006). A drug-based intervention study on the importance of buffaloes for human *Schistosoma japonicum* infection around Poyang Lake, People's Republic of China. *American Journal of Tropical Medicine and Hygiene* **74**, 335–341.

Guo, D., Wang, H., Zeng, D., Li, X., Fan, X. and Li, Y. (2011). Vaccine potential of hemocyanin from *Oncomelania hupensis* against *Schistosoma japonicum*. *Parasitology International* **60**, 242–246.

Guo, J. J., Zheng, H. J., Xu, J., Zhu, X. Q., Wang, S. Y. and Xia, C. M. (2012). Sensitive and specific target sequences selected from retrotransposons of *Schistosoma japonicum* for the diagnosis of schistosomiasis. *PLoS Neglected Tropical Diseases* **6**, e1579.

Han, H., Peng, J., Hong, Y., Zhang, M., Han, Y., Liu, D., Fu, Z., Shi, Y., Xu, J., Tao, J. and Lin, J. (2013). MicroRNA expression profile in different tissues of BALB/c mice in the early phase of *Schistosoma japonicum* infection. *Molecular and Biochemical Parasitology* **188**, 1–9.

Han, Y., Zhang, M., Hong, Y., Zhu, Z., Li, D., Li, X., Fu, Z. and Lin, J. (2012). Characterization of thioredoxin glutathione reductase in *Schiotosoma japonicum*. *Parasitology International* **61**, 475–480.

He, X., Sai, X., Chen, C., Zhang, Y., Xu, X., Zhang, D. and Pan, W. (2013). Host serum miR-223 is a potential new biomarker for *Schistosoma japonicum* infection and the response to chemotherapy. *Parasites and Vectors* **6**, 272.

He, S., Yang, L., Lv, Z., Hu, W., Cao, J., Wei, J., Sun, X., Yang, J., Zheng, H. and Wu, Z. (2010). Molecular and functional characterization of a mortalin-like protein from *Schistosoma japonicum* (SjMLP/hsp70) as a member of the HSP70 family. *Parasitology Research* **107**, 955–966.

Hesse, M., Cheever, A. W., Jankovic, D. and Wynn, T. A. (2000). NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *American Journal of Pathology* **157**, 945–955.

Hesse, M., Modolell, M., La Flamme, A. C., Schito, M., Fuentes, J. M., Cheever, A. W., Pearce, E. J. and Wynn, T. A. (2001). Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. *Journal of Immunology* **167**, 6533–6544.

Hong, Y., Han, Y., Fu, Z., Han, H., Qiu, C., Zhang, M., Yang, J., Shi, Y., Li, X. and Lin, J. (2013). Characterization and expression of the *Schistosoma japonicum* thioredoxin peroxidase-2 gene. *Journal of Parasitology* **99**, 68–76.

Hong, Y., Han, H., Peng, J., Li, Y., Shi, Y., Fu, Z., Liu, J., Lin, J. and Li, X. (2010). *Schistosoma japonicum*: cloning, expression and characterization of a gene encoding the alpha5-subunit of the proteasome. *Experimental Parasitology* **126**, 517–525.

Horsnell, W. G. and Brombacher, F. (2010). Genes associated with alternatively activated macrophages discretely regulate helminth infection and pathogenesis in experimental mouse models. *Immunobiology* **215**, 704–708.

Hou, X., Yu, F., Man, S., Huang, D., Zhang, Y., Liu, M., Ren, C. and Shen, J. (2012). Negative regulation of *Schistosoma japonicum* egg-induced liver fibrosis by natural killer cells. *PLoS Neglected Tropical Diseases* **6**, e1456.

Hsu, S. Y., Xu, S. T., He, Y. X., Shi, F. H., Shen, W., Hsu, H. F., Osborne, J. W. and Clarke, W. R. (1984). Vaccination of bovines against schistosomiasis japonica with highly irradiated schistosomula in China. *American Journal of Tropical Medicine and Hygiene* **33**, 891–898.

Hu, W., Yan, Q., Shen, D. K., Liu, F., Zhu, Z. D., Song, H. D., Xu, X. R., Wang, Z. J., Rong, Y. P., Zeng, L. C., Wu, J., Zhang, X., Wang, J. J., Xu, X. N., Wang, S. Y., Fu, G., Zhang, X. L., Wang, Z. Q., Brindley, P. J., McManus, D. P., Xue, C. L., Feng, Z., Chen, Z. and Han, Z. G. (2003). Evolutionary and biomedical implications of a *Schistosoma japonicum* complementary DNA resource. *Nature Genetics* **35**, 139–147.

Hu, Y., Lu, W., Shen, Y., Xu, Y., Yuan, Z., Zhang, C., Wu, J., Ni, Y., Liu, S. and Cao, J. (2012). Immune changes of *Schistosoma japonicum* infections in various rodent disease models. *Experimental Parasitology* **131**, 180–189.

Hua, W. Q., Yu, C. X., Yin, X. R. and Qian, C. Y. (2011). Application of F-ELISA for immunodiagnosis of schistosomiasis japonica. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* **23**, 158–162.

Huang, H., Liu, F., Huang, S., Yuan, S., Liao, B., Yi, S., Zeng, Y. and Chu, P. K. (2012). Sensitive and simultaneous detection of different disease markers using multiplexed gold nanorods. *Analytica Chimica Acta* **755**, 108–114.

Kallestrup, P., Zinyama, R., Gomo, E., Butterworth, A. E., van Dam, G. J., Gerstoft, J., Erikstrup, C. and Ullum, H. (2006). Schistosomiasis and HIV in rural Zimbabwe: efficacy of treatment of schistosomiasis in individuals with HIV coinfection. *Clinical Infectious Diseases* **42**, 1781–1789.

Kanamura, H. Y., Hancock, K., Rodrigues, V. and Damian, R. T. (2002). *Schistosoma mansoni* heat shock protein 70 elicits an early humoral immune response in *S. mansoni* infected baboons. *Memorias do Instituto Oswaldo Cruz* **97**, 711–716.

Kane, C. M., Cervi, L., Sun, J., McKee, A. S., Masek, K. S., Shapira, S., Hunter, C. A. and Pearce, E. J. (2004). Helminth antigens modulate TLR-initiated dendritic cell activation. *Journal of Immunology* **173**, 7454–7461.

Kariuki, T. M., Van Dam, G. J., Deelder, A. M., Farah, I. O., Yole, D. S., Wilson, R. A. and Coulson, P. S. (2006). Previous or ongoing schistosome infections do not compromise the efficacy of the attenuated cercaria vaccine. *Infection and Immunity* **74**, 3979–3986.

Karl, S., Gutierrez, L., Lucyk-Maurer, R., Kerr, R., Candido, R. R., Toh, S. Q., Saunders, M., Shaw, J. A., Suvorova, A., Hofmann, A., House, M. J., Woodward, R. C., Graeff-Teixera, C., St Pierre, T. G. and Jones, M. K. (2013). The iron distribution and magnetic properties of schistosome eggshells: implications for improved diagnostics. *PLoS Neglected Tropical Diseases* **7**, e2219.

Karmakar, S., Zhang, W., Ahmad, G., Torben, W., Alam, M. U., Le, L., Damian, R. T., Wolf, R. F., White, G. L., Carey, D. W., Carter, D., Reed, S. G. and Siddiqui, A. A. (2014). Use of an Sm-p80-based therapeutic vaccine to kill established adult Schistosome parasites in chronically infected baboons. *Journal of Infectious Diseases* **209**, 1929–1940.

King, C. H., Dickman, K. and Tisch, D. J. (2005). Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **365**, 1561–1569.

Kosinski, K. C., Adjei, M. N., Bosompem, K. M., Crocker, J. J., Durant, J. L., Osabutey, D., Plummer, J. D., Stadecker, M. J., Wagner, A. D., Woodin, M. and Gute, D. M. (2012). Effective control of *Schistosoma haematobium* infection in a Ghanaian community following installation of a water recreation area. *PLoS Neglected Tropical Diseases* **6**, e1709.

Lebens, M., Sun, J. B., Czerkinsky, C. and Holmgren, J. (2004). Current status and future prospects for a vaccine against schistosomiasis. *Expert Review of Vaccines* **3**, 315–328.

Lei, J. H., Su, B. T., Xu, H., Shen, J. L., Guan, X. H., Feng, Z. Q., Li, Y. L., Xu, M. X. and Liu, W. Q. (2011). Evaluation of an IgY-based immunomagnetic enzyme-linked immunosorbent assay system for detection of circulating *Schistosoma japonicum* antigen in serum samples from patients in China. *American Journal of Tropical Medicine and Hygiene* **85**, 1054–1059.

Lei, J. H., Guan, F., Xu, H., Chen, L., Su, B. T., Zhou, Y., Wang, T., Li, Y. L. and Liu, W. Q. (2012). Application of an immunomagnetic bead ELISA based on IgY for detection of circulating antigen in urine of mice infected with *Schistosoma japonicum*. *Veterinary Parasitology* **187**, 196–202.

Li, G. F., Wang, Y., Zhang, Z. S., Wang, X. J., Ji, M. J., Zhu, X., Liu, F., Cai, X. P., Wu, H. W. and Wu, G. L. (2005). Identification of immunodominant Th1-type T cell epitopes from *Schistosoma japonicum* 28 kDa glutathione-S-transferase, a vaccine candidate. *Acta Biochimca et Biophysica Sinica (Shanghai)* **37**, 751–758.

Li, Y. S., McManus, D. P., Lin, D. D., Williams, G. M., Harn, D. A., Ross, A. G., Feng, Z. and Gray, D. J. (2014*a*). The *Schistosoma japonicum* self-cure phenomenon in water buffaloes: potential impact on the control and elimination of schistosomiasis in China. *International Journal for Parasitology* **44**, 167–171.

Li, Y. S., McManus, D. P., Lin, D. D., Williams, G. M., Harn, D. A., Ross, A. G., Feng, Z. and Gray, D. J. (2014*b*). The *Schistosoma japonicum* self-cure phenomenon in water buffaloes: potential impact on the control and elimination of schistosomiasis in China. *International Journal for Parasitology* **44**, 167–171.

Liang, Y. J., Luo, J., Lu, Q., Zhou, Y., Wu, H. W., Zheng, D., Ren, Y. Y., Sun, K. Y., Wang, Y. and Zhang, Z. S. (2012). Gene profile of chemokines on hepatic stellate cells of schistosome-infected mice and antifibrotic roles of CXCL9/10 on liver non-parenchymal cells. *PLoS ONE* **7**, e42490.

Lier, T., Johansen, M. V., Hjelmevoll, S. O., Vennervald, B. J. and Simonsen, G. S. (2008). Real-time PCR for detection of low intensity *Schistosoma japonicum* infections in a pig model. *Acta Tropica* **105**, 74–80.

Lin, D., Tian, F., Wu, H., Gao, Y., Wu, J., Zhang, D., Ji, M., McManus, D. P., Driguez, P. and Wu, G. (2011*a*). Multiple vaccinations with UV-attenuated cercariae in pig enhance protective immunity against *Schistosoma japonicum* infection as compared to single vaccination. *Parasites and Vectors* **4**, 103.

Lin, D. D., Liu, Y. M., Hu, F., Li, Y. F., Tao, B., Yuan, M., Xie, S. Y., Huang, M. J., Jiang, Q. L., Li, J. Y., Gao, Z. L. and Wang, J. M. (2011*b*). Evaluation on application of common diagnosis methods for schistosomiasis japonica in endemic areas of China. III. Analysis and evaluation of underestimation of prevalence of *Schistosoma japonicum* infection by routine Kato-Katz technique. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* **23**, 642–647.

Liu, J. M., Cai, X. Z., Lin, J. J., Fu, Z. Q., Yang, G. Z., Shi, F. H., Cai, Y. M., Shen, W., Taylor, M. G. and Wu, X. F. (2004). Gene cloning, expression and vaccine testing of *Schistosoma japonicum* SjFABP. *Parasite Immunology* **26**, 351–358.

Liu, J. L., Fontaine, J., Capron, A. and Grzych, J. M. (1996). Ultrastructural localization of Sm28 GST protective antigen in *Schistosoma mansoni* adult worms. *Parasitology* **113** (Pt 4), 377–391.

Liu, P., Shi, Y., Yang, Y., Cao, Y., Shi, Y., Li, H., Liu, J., Lin, J. and Jin, Y. (2012). *Schistosoma japonicum* UDP-glucose 4-epimerase protein is located on the tegument and induces moderate protection against challenge infection. *PLoS One* **7**, e42050.

Liu, S., Song, G., Xu, Y., Yang, W. and McManus, D. P. (1995). Immunization of mice with recombinant Sjc26GST induces a pronounced anti-fecundity effect after experimental infection with Chinese *Schistosoma japonicum*. *Vaccine* **13**, 603–607.

Liu, J., Wang, J., Wang, S., Xu, B., Liu, X., Wang, X. and Hu, W. (2013). Molecular cloning and characterization of *Schistosoma japonicum* aldose reductase. *Parasitology Research* **112**, 549–558.

Loukas, A., Jones, M. K., King, L. T., Brindley, P. J. and McManus, D. P. (2001). Receptor for Fc on the surfaces of schistosomes. *Infection and Immunity* **69**, 3646–3651.

Loukas, A., Tran, M. and Pearson, M. S. (2007). Schistosome membrane proteins as vaccines. *International Journal for Parasitology* **37**, 257–263.

Lu, Y., Xu, B., Ju, C., Mo, X., Chen, S., Feng, Z., Wang, X. and Hu, W. (2012*a*). Identification and profiling of circulating antigens by screening with the sera from schistosomiasis japonica patients. *Parasites and Vectors* **5**, 115.

Lu, Y., Xu, B., Ju, C., Mo, X. J., Chen, S. B., Feng, Z., Wang, X. N. and Hu, W. (2012*b*). Cloning, expression and immunodiagnostic analysis of *Schistosoma japonicum* calcium-binding EF-hand domain containing protein. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)* **30**, 165–169.

Luo, X., Xie, H., Chen, D., Yu, X., Wu, F., Li, L., Wu, C. and Huang, J. (2014). Changes in NK and NKT cells in mesenteric lymph nodes after a *Schistosoma japonicum* infection. *Parasitology Research* **113**, 1001–1009.

Luo, X. P., Chen, D. H., Xie, H. Y., Gao, Z. Y., Fang, H. L. and Huang, J. (2012). Immune response of Th17 cells in mesenteric lymph node of mice infected by *Schistosoma japonicum*. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)*, **30**, 258–261, 267.

McKee, A. S. and Pearce, E. J. (2004). CD25 + CD4 + cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *Journal of Immunology* **173**, 1224–1231.

https://doi.org/10.1017/S0031182014001310 Published online by Cambridge University Press

McManus, D. P. and Dalton, J. P. (2006). Vaccines against the zoonotic trematodes *Schistosoma japonicum*, Fasciola hepatica and Fasciola gigantica. *Parasitology* **133**(Suppl), S43–S61.

McManus, D. P. and Loukas, A. (2008). Current status of vaccines for schistosomiasis. *Clinical Microbiology Reviews* **21**, 225–242.

McManus, D. P., Gray, D. J., Li, Y., Feng, Z., Williams, G. M., Stewart, D., Rey-Ladino, J. and Ross, A. G. (2010). Schistosomiasis in the People's Republic of China: the era of the Three Gorges Dam. *Clinical Microbiology Reviews* **23**, 442–466.

McManus, D. P., Wong, J. Y., Zhou, J., Cai, C., Zeng, Q., Smyth, D., Li, Y., Kalinna, B. H., Duke, M. J. and Yi, X. (2001). Recombinant paramyosin (rec-Sj-97) tested for immunogenicity and vaccine efficacy against *Schistosoma japonicum* in mice and water buffaloes. *Vaccine* **20**, 870–878.

McWilliam, H. E., Piedrafita, D., Li, Y., Zheng, M., He, Y., Yu, X., McManus, D. P. and Meeusen, E. N. (2013). Local immune responses of the Chinese water buffalo, *Bubalus bubalis*, against *Schistosoma japonicum* larvae: crucial insights for vaccine design. *PLoS Neglected Tropical Diseases* **7**, e2460.

Mentink-Kane, M. M. and Wynn, T. A. (2004). Opposing roles for IL-13 and IL-13 receptor alpha 2 in health and disease. *Immunological Reviews* **202**, 191–202.

Mo, A. X., Agosti, J. M., Walson, J. L., Hall, B. F. and Gordon, L. (2014). Schistosomiasis elimination strategies and potential role of a vaccine in achieving global health goals. *American Journal of Tropical Medicine and Hygiene* **90**, 54–60.

Modolell, M., Corraliza, I. M., Link, F., Soler, G. and Eichmann, K. (1995). Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *European Journal of Immunology* **25**, 1101–1104.

Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J. A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S. Y., Ali, M. K., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Bahalim, A. N., Barker-Collo, S., Barrero, L. H., Bartels, D. H., Basanez, M. G., Baxter, A., Bell, M. L., Benjamin, E. J., *et al.* (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2197–2223.

Obeng, B. B., Aryeetey, Y. A., de Dood, C. J., Amoah, A. S., Larbi, I. A., Deelder, A. M., Yazdanbakhsh, M., Hartgers, F. C., Boakye, D. A., Verweij, J. J., van Dam, G. J. and van Lieshout, L. (2008). Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. *Annals of Tropical Medicine and Parasitology* **102**, 625–633.

Ohta, N., Kumagai, T., Maruyama, H., Yoshida, A., He, Y. and Zhang, R. (2004). Research on calpain of *Schistosoma japonicum* as a vaccine candidate. *Parasitology International* **53**, 175–181.

Pearce, E. J. and MacDonald, A. S. (2002). The immunobiology of schistosomiasis. *Nature Reviews: Immunology* **2**, 499–511.

Peng, J., Han, H., Hong, Y., Wang, Y., Guo, F., Shi, Y., Fu, Z., Liu, J., Cheng, G. and Lin, J. (2010). Cloning and expressing of cyclophilin B gene from *Schistosoma japonicum* and the analysis of immunoprotective effect. *Sheng Wu Gong Cheng Xue Bao* **26**, 317–323.

Qian, C. Y., Huang, B., Yu, C. X., Zhang, J., Yin, X. R., Wang, J., Song, L. J., Zhang, W. and Ke, X. D. (2011). Detection of the circulating antigen 14-3-3 protein of *Schistosoma japonicum* by time-resolved fluoroimmunoassay in rabbits. *Parasites and Vectors* **4**, 95.

Qiu, C., Liu, S., Hong, Y., Fu, Z., Wei, M., Ai, D. and Lin, J. (2012). Molecular characterization of thyroid hormone receptor beta from *Schistosoma japonicum* and assessment of its potential as a vaccine candidate antigen against schistosomiasis in BALB/c mice. *Parasites and Vectors* **5**, 172.

Rabello, A. and Enk, M. (2006). Progress towards the detection of schistosomiasis. In *Report of the Scientific Working Group Meeting on Schistosomiasis for the Special Programme for Research and Training in Tropical Diseases* (ed. WHO), pp. 67–71. WHO, Geneva.

Reid, G. D., Sturrock, R. F., Harrison, R. A. and Tarara, R. P. (1995). *Schistosoma haematobium* in the baboon (Papio anubis): assessment of protection levels against either a single mass challenge or repeated trickle challenges after vaccination with irradiated schistosomula. *Journal of Helminthology*, **69**, 139–147.

Riner, D. K., Ferragine, C. E., Maynard, S. K. and Davies, S. J. (2013). Regulation of innate responses during pre-patent schistosome infection provides an immune environment permissive for parasite development. *PLoS Pathogens* **9**, e1003708.

Ross, A. G., Bartley, P. B., Sleigh, A. C., Olds, G. R., Li, Y., Williams, G. M. and McManus, D. P. (2002). Schistosomiasis. *New England Journal of Medicine* **346**, 1212–1220.

Sabin, E. A., Kopf, M. A. and Pearce, E. J. (1996). *Schistosoma mansoni* egg-induced early IL-4 production is dependent upon IL-5 and eosinophils. *Journal of Experimental Medicine*, **184**, 1871–1878.

Schramm, G., Falcone, F. H., Gronow, A., Haisch, K., Mamat, U., Doenhoff, M. J., Oliveira, G., Galle, J., Dahinden, C. A. and Haas, H. (2003). Molecular characterization of an interleukin-4-inducing factor from *Schistosoma mansoni* eggs. *Journal of Biological Chemistry* **278**, 18384–18392.

Seto, E. Y., Remais, J. V., Carlton, E. J., Wang, S., Liang, S., Brindley, P. J., Qiu, D., Spear, R. C., Wang, L. D., Wang, T. P., Chen, H. G., Dong, X. Q., Wang, L. Y., Hao, Y., Bergquist, R. and Zhou, X. N. (2011). Toward sustainable and comprehensive control of schistosomiasis in China: lessons from Sichuan. *PLoS Neglected Tropical Diseases* **5**, e1372.

Shi, Y. E., Jiang, C. F., Han, J. J., Li, Y. L. and Ruppel, A. (1990). *Schistosoma japonicum*: an ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes. *Experimental Parasitology*, **71**, 100–106.

Shi, F., Zhang, Y., Ye, P., Lin, J., Cai, Y., Shen, W., Bickle, Q. D. and Taylor, M. G. (2001). Laboratory and field evaluation of *Schistosoma japonicum* DNA vaccines in sheep and water buffalo in China. *Vaccine* **20**, 462–467.

Shuxian, L., Yongkang, H., Guangchen, S., Xing-song, L., Yuxin, X. and McManus, D. P. (1997). Anti-fecundity immunity to *Schistosoma japonicum* induced in Chinese water buffaloes (*Bos buffelus*) after vaccination with recombinant 26 kDa glutathione-S-transferase (reSjc26GST). *Veterinary Parasitology* **69**, 39–47.

Siddiqui, A. A., Pinkston, J. R., Quinlin, M. L., Saeed, Q., White, G. L., Shearer, M. H. and Kennedy, R. C. (2005). Characterization of the immune response to DNA vaccination strategies for schistosomiasis candidate antigen, Sm-p80 in the baboon. *Vaccine* **23**, 1451–1456.

Siddiqui, A. A., Siddiqui, B. A. and Ganley-Leal, L. (2011). Schistosomiasis vaccines. *Human Vaccines* **7**, 1192–1197.

Sun, L. P., Wang, W., Liang, Y. S., Tian, Z. X., Hong, Q. B., Yang, K., Yang, G. J., Dai, J. R. and Gao, Y. (2011). Effect of an integrated control strategy for schistosomiasis japonica in the lower reaches of the Yangtze River, China: an evaluation from 2005 to 2008. *Parasites and Vectors* **4**, 243.

Tallima, H., Salah, M., Guirguis, F. R. and El Ridi, R. (2009). Transforming growth factor-beta and Th17 responses in resistance to primary murine *Schistosomiasis mansoni*. *Cytokine* **48**, 239–245.

Tang, H., Ming, Z., Liu, R., Xiong, T., Grevelding, C. G., Dong, H. and Jiang, M. (2013). Development of adult worms and granulomatous pathology are collectively regulated by T- and B-cells in mice infected with *Schistosoma japonicum*. *PLoS ONE* **8**, e54432.

Taylor, M. G., Huggins, M. C., Shi, F., Lin, J., Tian, E., Ye, P., Shen, W., Qian, C. G., Lin, B. F. and Bickle, Q. D. (1998). Production and testing of *Schistosoma japonicum* candidate vaccine antigens in the natural ovine host. *Vaccine* **16**, 1290–1298.

Tian, F., Lin, D., Wu, J., Gao, Y., Zhang, D., Ji, M. and Wu, G. (2010). Immune events associated with high level protection against *Schistosoma japonicum* infection in pigs immunized with UV-attenuated cercariae. *PLoS ONE* **5**, e13408.

Vercruysse, J., Schetters, T. P., Knox, D. P., Willadsen, P. and Claerebout, E. (2007). Control of parasitic disease using vaccines: an answer to drug resistance? *Revue Scientifique et Technique, Office International des Epizooties* **26**, 105–115.

Wang, C., Chen, L., Yin, X., Hua, W., Hou, M., Ji, M., Yu, C. and Wu, G. (2011*a*). Application of DNA-based diagnostics in detection of schistosomal DNA in early infection and after drug treatment. *Parasites and Vectors* **4**, 164.

Wang, J., Yu, C. X., Yin, X. R., Zhang, W., Qian, C. Y., Song, L. J., Ke, X. D., Xu, Y. L., He, W. and Cao, G. Q. (2011*b*). Monitoring specific antibody responses against the hydrophilic domain of the 23 kDa membrane protein of *Schistosoma japonicum* for early detection of infection in sentinel mice. *Parasites and Vectors* **4**, 172.

Wang, S. P., Chen, X. C. and Gao, D. M. (2009). Research progress on schistosome vaccine and prospect of its application in China. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)* **27**, 402–411.

Wang, L., Liu, W., Yang, M., Peng, D. and Chen, L. (2013). Development of a *Streptococcus gordonii* vaccine strain expressing *Schistosoma japonicum* Sj-F1 and evaluation of using this strain for intranasal immunization in mice. *Parasitology Research* **112**, 1701–1708.

Wang, J., Song, L. J., He, W., Yin, X. R., Qian, C. Y., Zhang, W., Xu, Y. L., Cao, G. Q., Ke, X. D. and Yu, C. X. (2012). Identification of

early diagnostic molecules in soluble egg antigen of *Schistosoma japonicum* by MASS. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* **24**, 132–136.

Wang, T., Zhang, S., Wu, W., Zhang, G., Lu, D., Ornbjerg, N. and Johansen, M. V. (2006*a*). Treatment and reinfection of water buffaloes and cattle infected with *Schistosoma japonicum* in Yangtze River Valley, Anhui province, China. *Journal of Parasitology* **92**, 1088–1091.

Wang, X. H., Wu, X. H. and Zhou, X. N. (2006*b*). Bayesian estimation of community prevalences of *Schistosoma japonicum* infection in China. *International Journal for Parasitology* **36**, 895–902.

Wen, X., He, L., Chi, Y., Zhou, S., Hoellwarth, J., Zhang, C., Zhu, J., Wu, C., Dhesi, S., Wang, X., Liu, F. and Su, C. (2011). Dynamics of Th17 cells and their role in *Schistosoma japonicum* infection in C57BL/6 mice. *PLoS Neglected Tropical Diseases* **5**, e1399.

Wichmann, D., Panning, M., Quack, T., Kramme, S., Burchard, G. D., Grevelding, C. and Drosten, C. (2009). Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. *PLoS Neglected Tropical Diseases* **3**, e422.

Williams, G. M., Sleigh, A. C., Li, Y., Feng, Z., Davis, G. M., Chen, H., Ross, A. G., Bergquist, R. and McManus, D. P. (2002). Mathematical modelling of schistosomiasis japonica: comparison of control strategies in the People's Republic of China. *Acta Tropica* **82**, 253–262.

Wilson, M. S., Mentink-Kane, M. M., Pesce, J. T., Ramalingam, T. R., Thompson, R. and Wynn, T. A. (2007). Immunopathology of schistosomiasis. *Immunology and Cell Biology* **85**, 148–154.

Wu, Z., Liu, S., Zhang, S., Tong, H., Gao, Z., Liu, Y., Lin, D., Liu, Z., Wu, G., Yi, H., Song, G. and Xu, Y. (2004). Persistence of the protective immunity to *Schistosoma japonicum* in Chinese yellow cattle induced by recombinant 26 kDa glutathione-S-transferase (reSjc26GST). *Veterinary Parasitology* **123**, 167–177.

Wu, Z. D., Lu, Z. Y. and Yu, X. B. (2005). Development of a vaccine against *Schistosoma japonicum* in China: a review. *Acta Tropica* **96**, 106–116.

Wu, X., Zhao, B., Hong, Y., Li, X., Peng, J., Zhang, J., Wang, F., Shi, Y., Fu, Z. and Lin, J. (2012). Characterization of *Schistosoma japonicum* estrogen-related receptor beta like 1 and immunogenicity analysis of the recombinant protein. *Experimental Parasitology*, **131**, 383–392.

Xiong, Y., Zhang, M., Hong, Y., Wei, M., Ai, D., Meng, P., Han, Y., Fu, Z., Shi, Y., Yang, J. and Lin, J. (2013). Characterization analysis of plasma membrane repair relative gene myoferlin. *PLoS One*, **8**, e66396.

Xu, J., Feng, T., Lin, D. D., Wang, Q. Z., Tang, L., Wu, X. H., Guo, J. G., Peeling, R. W. and Zhou, X. N. (2011*a*). Performance of a dipstick dye immunoassay for rapid screening of *Schistosoma japonicum* infection in areas of low endemicity. *Parasites and Vectors* **4**, 87.

Xu, B., Ju, C., Lu, Y., Mo, X. J., Feng, Z., Xu, X. N. and Hu, W. (2011*b*). Cloning and expression of metalloprotease gene from *Schistosoma japonicum* and its immunoprotective efficiency. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)* **29**, 81–86.

Xu, B., Gordon, C. A., Hu, W., McManus, D. P., Chen, H. G., Gray, D. J., Ju, C., Zeng, X. J., Gobert, G. N., Ge, J., Lan, W. M., Xie, S. Y., Jiang, W. S., Ross, A. G., Acosta, L. P., Olveda, R. and Feng, Z. (2012). A novel procedure for precise quantification of *Schistosoma japonicum* eggs in bovine feces. *PLoS Neglected Tropical Diseases* **6**, e1885.

Xu, J., Rong, R., Zhang, H. Q., Shi, C. J., Zhu, X. Q. and Xia, C. M. (2010). Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). *International Journal for Parasitology* **40**, 327–331.

Xu, J., Liu, A. P., Guo, J. J., Wang, B., Qiu, S. J., Sun, H., Guan, W., Zhu, X. Q., Xia, C. M. and Wu, Z. D. (2013). The sources and metabolic dynamics of *Schistosoma japonicum* DNA in serum of the host. *Parasitology Research* **112**, 129–133.

Xu, X., Zhang, Y., Lin, D., Zhang, J., Xu, J., Liu, Y. M., Hu, F., Qing, X., Xia, C. and Pan, W. (2014). Serodiagnosis of *Schistosoma japonicum* infection: genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. *Lancet Infectious Diseases* **14**, 489–497.

Yang, J., Fu, Z., Feng, X., Shi, Y., Yuan, C., Liu, J., Hong, Y., Li, H., Lu, K. and Lin, J. (2012). Comparison of worm development and host immune responses in natural hosts of *Schistosoma japonicum*, yellow cattle and water buffalo. *BMC Veterinary Research* **8**, 25.

You, H., Zhang, W., Moertel, L., McManus, D. P. and Gobert, G. N. (2009). Transcriptional profiles of adult male and female *Schistosoma japonicum* in response to insulin reveal increased expression of genes involved in growth and development. *International Journal for Parasitology* **39**, 1551–1559.

You, H., Gobert, G. N., Duke, M. G., Zhang, W., Li, Y., Jones, M. K. and McManus, D. P. (2012). The insulin receptor is a transmission blocking veterinary vaccine target for zoonotic *Schistosoma japonicum*. *International Journal for Parasitology* **42**, 801–807.

You, H., Stephenson, R. J., Gobert, G. N. and McManus, D. P. (2014). Revisiting glucose uptake and metabolism in schistosomes: new molecular insights for improved schistosomiasis therapies. *Frontiers in Genetics* **5**, 176.

Young, N. D., Jex, A. R., Li, B., Liu, S., Yang, L., Xiong, Z., Li, Y., Cantacessi, C., Hall, R. S., Xu, X., Chen, F., Wu, X., Zerlotini, A., Oliveira, G., Hofmann, A., Zhang, G., Fang, X., Kang, Y., Campbell, B. E., Loukas, A., Ranganathan, S., Rollinson, D., Rinaldi, G., Brindley, P. J., Yang, H., Wang, J., Wang, J. and Gasser, R. B. (2012). Whole-genome sequence of *Schistosoma haematobium*. *Nature Genetics* **44**, 221–225.

Yu, L. L., Ding, J. Z., Wen, L. Y., Lou, D., Yan, X. L., Lin, L. J., Lu, S. H., Lin, D. D. and Zhou, X. N. (2011). Development of a rapid dipstick with latex immunochromatographic assay (DLIA) for diagnosis of schistosomiasis japonica. *Parasites and Vectors* **4**, 157.

Yu, X. L., He, Y. K., Xiong, T., Zhao, Y. Q., Shi, M. Z., Zhou, J., Liu, Z. C., Luo, X. S., Fu, X., He, H. B., Harn, D. A. and Li, Y. S. (2006). Protective effects of co-immunization with SjCTPI-Hsp70 and interleukin-12 DNA vaccines against *Schistosoma japonicum* challenge infection in water buffalo. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)* **24**, 433–436.

Yu, Q., Yang, H., Feng, Y., Yang, X. and Zhu, Y. (2012*a*). Magnetic affinity enzyme-linked immunoassay based on recombinant 26 kDa glutathione-S-transferase for serological diagnosis of schistosomiasis japonica. *Acta Tropica* **124**, 199–202.

Yu, Q., Yang, H., Feng, Y., Zhu, Y. and Yang, X. (2012*b*). Magnetic affinity enzyme-linked immunoassay for diagnosis of *Schistosomiasis japonicum* in persons with low-intensity infection. *American Journal of Tropical Medicine and Hygiene* **87**, 689–693.

Yu, Q., Yang, H., Guan, F., Feng, Y., Yang, X. and Zhu, Y. (2014). Detection of IgG in sera of patients with schistosomiasis japonica by developing magnetic affinity enzyme-linked immunoassay based on recombinant 14-3-3 protein. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **108**, 37–41.

Zaccone, P., Fehervari, Z., Jones, F. M., Sidobre, S., Kronenberg, M., Dunne, D. W. and Cooke, A. (2003). *Schistosoma mansoni* antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *European Journal of Immunology*, **33**, 1439–1449.

Zhang, X., Gao, Y. N., Hou, M., Chen, L., Ji, M. J. and Wu, G. L. (2011). Antibody responses to leucine aminopeptidase in *Schistosoma japonicum* infection. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* **23**, 163–167.

Zhang, X. B., Hu, F., Xie, S. Y., Tao, B., Yuan, M., Liu, Y. M., Li, J. Y., Li, Z. J. and Lin, D. D. (2012*a*). Field application of antibody detection in a low transmission area of *Schistosoma japonicum*. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi (Chinese Journal of Parasitology and Parasitic Diseases)* **30**, 468–473.

Zhang, Y., Chen, L., Gao, W., Hou, X., Gu, Y., Gui, L., Huang, D., Liu, M., Ren, C., Wang, S. and Shen, J. (2012*b*). IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. *European Journal of Immunology* **42**, 1523–1535.

Zhang, Z., Xu, H., Gan, W., Zeng, S. and Hu, X. (2012*c*). *Schistosoma japonicum* calcium-binding tegumental protein SjTP22.4 immunization confers praziquantel schistosomulumicide and antifecundity effect in mice. *Vaccine* **30**, 5141–5150.

Zhang, M., Han, Y., Zhu, Z., Li, D., Hong, Y., Wu, X., Fu, Z. and Lin, J. (2012*d*). Cloning, expression, and characterization of *Schistosoma japonicum* tegument protein phosphodiesterase-5. *Parasitology Research* **110**, 775–786.

Zhang, R., Yoshida, A., Kumagai, T., Kawaguchi, H., Maruyama, H., Suzuki, T., Itoh, M., El-Malky, M. and Ohta, N. (2001). Vaccination with calpain induces a Th1-biased protective immune response against *Schistosoma japonicum*. *Infection and Immunity* **69**, 386–391.

Zhou, X. H., Wu, J. Y., Huang, X. Q., Kunnon, S. P., Zhu, X. Q. and Chen, X. G. (2010). Identification and characterization of *Schistosoma japonicum* Sjp40, a potential antigen candidate for the early diagnosis of schistosomiasis. *Diagnostic Microbiology and Infectious Disease* **67**, 337–345.

Zhou, Y. B., Yang, M. X., Wang, Q. Z., Zhao, G. M., Wei, J. G., Peng, W. X. and Jiang, Q. W. (2007). Field comparison of immunodiagnostic and parasitological techniques for the detection of Schistosomiasis japonica in the People's Republic of China. *American Journal of Tropical Medicine and Hygiene* **76**, 1138–1143.

Zhou, Y., Zheng, H., Chen, Y., Zhang, L., Wang, K., Guo, J., Huang, Z., Zhang, B., Huang, W., Jin, K., Dou, T., Hasegawa, M., Wang, L., Zhang, Y., Zhou, J., Tao, L., Cao, Z., Li, Y., Vinar, T., Brejova, B., Brown, D., Li, M., Miller, D. J., Blair, D., Zhong, Y., Chen, Z., Liu, F., Hu, W., Wang, Z. Q., Zhang, Q. H., *et al.* (2009). The *Schistosoma*

japonicum genome reveals features of host–parasite interplay. *Nature* **460**, 345–351.

Zhu, Y., Ren, J., Da'dara, A., Harn, D., Xu, M., Si, J., Yu, C., Liang, Y., Ye, P., Yin, X., He, W., Xu, Y., Cao, G. and Hua, W. (2004). The protective effect of a *Schistosoma japonicum* Chinese strain 23 kDa plasmid DNA vaccine in pigs is enhanced with IL-12. *Vaccine* **23**, 78–83.

Zhu, Y., Si, J., Ham, D. A., Yu, C., He, W., Hua, W., Yin, X., Liang, Y., Xu, M. and Xu, R. (2002). The protective immunity produced in infected C57BL/6 mice of a DNA vaccine encoding *Schistosoma japonicum* Chinese strain triose-phosphate isomerase. *Southeast Asian Journal of Tropical Medicine and Public Health* **33**, 207–213.

Zhu, Y., Si, J., Harn, D. A., Xu, M., Ren, J., Yu, C., Liang, Y., Yin, X., He, W. and Cao, G. (2006). *Schistosoma japonicum* triose-phosphate isomerase plasmid DNA vaccine protects pigs against challenge infection. *Parasitology* **132**, 67–71.

Zhu, Y. C. (2005). Immunodiagnosis and its role in schistosomiasis control in China: a review. *Acta Tropica* **96**, 130–136.

Zhu, Z., Fu, Z., Zhang, M., Han, Y., Hong, Y., Li, D., Zhao, Z., Shi, Y., Li, X. and Lin, J. (2012). Protective efficacy evaluation induced by recombinant protein LHD-Sj23-GST of *Schistosoma japonicum* emulsified with three different adjuvants. *Parasite Immunology* **34**, 341–344.

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