CHAPTER FOURTEEN

Development and Application of Diagnostics in the National Schistosomiasis Control Programme in The People's Republic of China

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Contents

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Abstract

Schistosomiasis, caused by *Schistosoma japonicum* infection to human, has a documented history of more than 2100 years in The People's Republic of China. In spite of great progress in controlling the disease, it is still one of the most serious parasitic diseases in the country. The study and use of diagnostic techniques play an important role in the targeting of chemotherapy that has been continuously applied in the national schistosomiasis control programme for several decades. This paper reviews the development and application of parasitological, immunodiagnostic and molecular diagnostic technology for *S. japonicum* in The People's Republic of China with a brief mention of diagnostic imagery, such as ultrasound and radiology. When analysing the efficacy and performance characteristics of the main diagnostic techniques in current use, it becomes apparent that approaches that worked well in the past are less suitable now as successful control has shifted the endemic situation towards control and interruption of transmission. The conclusion is that a mutable approach must be adopted choosing the most appropriate diagnostic technique for each control stage (and area), thus modifying the methodology according to the prevailing diagnostic needs in terms of sensitivity and specificity.

1. INTRODUCTION

Schistosomiasis in The People's Republic of China is confined to infection by *Schistosoma japonicum*. The first report of a human case where the clinical symptoms were directly connected with this parasite was made by an American physician working in The People's Republic of China (Chen, 2014; Logan, 1905). After that, only sporadic reports are available in English, but in the mid-1950s, there were more than 11 million known infected cases (Chen and Feng, 1999; Mao and Shao, 1982; Xu et al., 2016). Thanks to sustained, strong control efforts started then, both prevalence rates and the intensity of infection in the endemic areas have dramatically declined since then resulting in a drop to an estimated 185,000 people in 2013 (Lei et al., 2014; Wang, 2006). According to the criteria for schistosomiasis control and elimination issued by the Chinese Ministry of Health, the stages for the national schistosomiasis control programme are classified according to the prevailing prevalence level, ie, morbidity control stage when prevalence is over than 5%, infection control stage when prevalence at 1−5%, and transmission control stage when prevalence less than 1%, while absence of local cases for 5 years signifies transmission interruption

and the continued absence of cases for another 5 years means that elimination has been achieved (Zhou et al., 2011; Zhu et al., 2016). Case finding and treatment, assessment of morbidity as well as evaluation of control strategies all build on the results of diagnostic tests, so it can emphatically be said that diagnosis is the essential basis of treatment and central to the control of schistosomiasis (Collins et al., 2012; Feldmeier and Poggensee, 1993).

The current, widely used diagnostic methodology can be divided into parasitological techniques (detection of parasite eggs or miracidia in the faeces) and immunologic approaches (detection of specific antibodies or circulating schistosome antigens). Parasitological techniques such as the Kato-Katz thick-smear method and the miracidium hatching technique are used to determine if there is infection and usually serve as reference to evaluate other diagnostic tools (Bergquist et al., 2009; Wu, 2002). Indirect approaches including immunodiagnostic techniques have superior sensitivity and are rapid as well as affordable. Antibody-based immunoassays are useful for large-scale screening but positive titres abate only slowly making it impossible to differentiate between current and cured infections. On the other hand, antibody techniques are highly effective for surveying areas from where infection has been eliminated (Hillyer et al., 1999). The detection of circulating antigens (CAg) is not only the most reliable technique (if the latest, high-sensitivity techniques are used — see the later discussion), but also the most direct one as the amount of released antigens from the adult worms in the host changes in direct relation to a change in the worm burden, while the excretion of eggs from the infected host is somewhat uneven (Engels et al., 1996), a variable that would be even more pronounced in the case of *S. japonicum* as this species tends to release its eggs in aggregates. In addition, the development of molecular biotechnology, including the polymerase chain reaction (PCR) and the loop-mediated isothermal amplification (LAMP), both based on the gene cloning amplification, has been developed and successfully transferred from the laboratory to the field (Lier et al., 2009; Xia et al., 2009; Xu et al., 2010, 2015).

Integration of diagnostic tools into national control programmes requires collaboration between research laboratories, epidemiologists and control programme managers, defining assays in terms of sensitivity, specificity and predictive values (Dabo et al., 2015). Big differences exist between different endemic areas and stages of control in terms of prevalence and infection intensity. Diagnostic tools and strategies should therefore vary according to the perceived prevalence level and attention should be paid to the prevailing socioeconomic situation. This article reviews the development and application of parasitological, immunodiagnostic and molecular diagnostic technology

for *S. japonicum* infection in The People's Republic of China with a brief mention of imaging techniques such as ultrasound and radiology. The currently most important diagnostic approaches useful for epidemiological survey against the backdrop of the historical record are also discussed.

2. HISTORICAL REVIEW OF DIAGNOSTIC TOOLS

2.1 Parasitological methods

The diagnosis of active *S. japonicum* infection consists of the detection of viable schistosome eggs in faeces or tissue biopsies. Direct smear, sedimentation, formol-ether concentration (Allen and Ridley, 1970), Kato-Katz thick smear (Katz et al., 1972), the hatching test (Qiu and Xue, 1990) and rectal biopsy can be used to diagnose schistosome infection. All these techniques require microscope and well-trained laboratory technicians. Although of little importance in the endemic areas, it should be pointed out that acute schistosomiasis, particularly in the very early stages and in 'immunologically naive' patients, could sometimes remain undiagnosed by the exclusive use of stool examination.

2.1.1 Stool examination

The detection of eggs or miracidia in stool samples is the traditional way to confirm an infection with *S. japonicum*. For quantification of faecal egg counts, the Kato-Katz method, originally developed in the mid-1950s by the Japanese researchers Kato and Miura (1954) and later standardized by the introduction of a 41.7 mg templates by Katz et al. (1972) in Brazil, is the most broadly used technique in epidemiological surveys pertaining to intestinal schistosomiasis. The number of eggs per gram (EPG) faeces is used as an index of the intensity of infection dividing cases into three categories: light infection (EPG <100), medium infection ($100 \leq$ EPG < 400) and heavy infection (EPG ≥ 400) as recommended by the World Health Organization (WHO, 2002). In spite of reduced utility with regard to light infections, the Kato-Katz thick stool smear remains the most commonly used technique for general prevalence mapping and field-based control of schistosomiasis.

Among other traditional tests, the hatching test, based on the positive phototrophic behaviour of schistosome miracidia, relies on the observation of released miracidia from parasite eggs hatched in faeces diluted in water and allowed to sediment (Qiu and Xue, 1990). The sensitivity of hatching

technique is supposedly higher than the Kato-Katz thick-smear method due to the large volume of faeces investigated (Zhu et al., 2014). Sometimes, the hatching technique, followed by microscopic examination of faecal sediment, can be implemented to improve sensitivity (Xu et al., 2007). However, the result is influenced by temperature, quality of the water added and examiner experience. The slight improvement of sensitivity achieved may not justify wider use due to the added time and cost required.

2.1.2 Tissue biopsy

When a schistosome infection is suspected but no eggs are found in faeces after multiple examinations, proctoscopy combined with rectal biopsy could be attempted to find eggs deposited in the intestinal mucosa. However, this is a clinical, hospital-based approach that does not play a role in large-scale, control schemes. In addition, the use of this approach in practice is limited as it has a low level of acceptance by patients and physicians alike due to associated complications, such as bleeding and the high possibility of sampling error (Yu et al., 1989).

2.2 Imaging techniques

Radiology, now generally replaced by computer tomography (CT) and magnetic resonance imaging (MRI) when searching for schistosome-induced hepatic or hepatosplenic enlargement, must be carried out in a hospital environment. Ultrasonography (US), on the other hand, represents a more versatile approach with several advantages. Not only can the technology be adopted for field use, but it is also less costly while still capable of demonstrating the classical features of periportal fibrosis (appearing as a netlike echogenic pattern in *S. japonicum* infection), hepatic granuloma and gallbladder thickening. The US approach is especially helpful for documenting advanced schistosomiasis. However, the technique is not only useful in the diagnosis and differential diagnosis of advanced schistosomiasis, but also for the guidance of treatment and evaluation of therapeutic effects and, furthermore, for risk prediction of portal hypertension and upper gastrointestinal haemorrhage (Wu et al., 2015). The introduction of portable US equipment broadened the applicability of diagnostic, imagery investigations in endemic community settings.

Although the US technology originated in 1940s and was not used for schistosomiasis investigations until the 1980s (Zhou et al., 2009), it has now overtaken the other imaging techniques in importance. However, the diagnostic characteristics of all three methodologies are similar in that

they are noninvasive and record the damage of intra-abdominal organs in a straightforward way. Imaging approaches are therefore particularly suitable for the diagnosis of ectopic schistosomiasis, eg, neuroschistosomiasis (Ross et al., 2012). Together with CT and MRI, US facilitates the detection of lesions, such as pathological enlargements and tissue oedema, which is suitable for location before clinical, invasive procedures (Fang and Xu, 2014; Li, 2015; Lv and Pei, 2007; Shen et al., 2012; Wang et al., 2014; Yang, 2015). While CT and MRI are not routinely used for schistosomiasis diagnosis in resource-poor areas for economic reasons and US is particularly useful in the field, all three techniques require highly qualified users with medical education and special training.

2.3 Immunological tests

Immunological tests for schistosomiasis diagnosis have a long history in The People's Republic of China. These tests were developed to detect the antibody or circulating schistosome antigens based on various labelling techniques emphasizing advantages such as high sensitivity, easy use and rapidity.

2.3.1 The intradermal test

The earliest test based on immune reactions in The People's Republic of China is the intradermal test (ID) introduced by Gan (1936), who adopted this approach from work described for other schistosome species. Antigens aimed at the detection of specific IgE antibodies were extracted from fresh or frozen adult worms, eggs, miracidia or cercaria and their diagnostic efficacy was evaluated in a trial. The advantages of ID, such as ease of use, low cost and high sensitivity (estimated at 90%) facilitated its implementation in the early 1950s in the national schistosomiasis control programme to investigate the distribution and prevalence of schistosomiasis japonica (Maegraith, 1958; Mao and Shao, 1982). However, the specificity was low, so it was soon replaced by other tests.

2.3.2 The circumoval precipitin test

This assay was originally described by Liu et al. (1958) and widely used in The People's Republic of China in the remaining part of last century. Circumoval precipitin test (COPT) substitutes intact fresh parasite eggs for the antigen preparation, which normally consists of schistosome eggs that have been lyophilized or subjected to heat and/or ultrasound. It has a high sensitivity (94–99%) and adequate specificity with low, false positive rates in healthy people from nonendemic areas (2–4%) (Li, 1991). After effective treatment

for 4 years, alternatively 3–8 years, the negative reversion rates were 82.5% and 80–83%, respectively (Li, 1991). However, with repeated treatments in the controlled areas leading to very low rates of prevalence and intensity of infection, the sensitivity of the test declined to 70–80% (Song et al., 2003). The technique is, however, comparatively complicated and time-consuming (48 h for recording the results in the laboratory) and requires microscopy, issues that limit its wider application in endemic areas these days.

2.3.3 The indirect haemagglutination assay

Soluble antigen preparations are necessary for tests relying on agglutination of microscopic particles, eg, sheep erythrocytes as used in the indirect haemagglutination assay (IHA), a test first employed for the diagnosis of *S. japonicum* in The People's Republic of China by Tao (Shi et al., 1980). Erythrocytes sensitized with schistosomes antigens in a first step will agglutinate when antibodies against such antigens in patients' serum samples combine with the antigens on the cell surfaces. After multiple modifications by Chinese scientists, the diagnostic efficacy and stability of IHA have been improved significantly. The sensitivity of IHA has thus reached 93–100%, while the false positive rate in healthy people from nonendemic areas have come down to 2–3%, and which have the diagnostic value in early infection (Chen et al., 2011a; Wang et al., 2010; Yang et al., 2009). After effective, periodical treatment for 3 years or more, most former schistosomiasis patients turn negative in the test. IHA remains a widely used general immunoassay in The People's Republic of China, secondary only to COPT in having been used for more than 50 years (Zhu et al., 2009).

2.3.4 The enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) detects the presence of specific antibodies (or antigens) in samples based on the specific combination of antibody and antigen made visible by the addition of secondary antibodies labelled with an enzyme-substrate (Engvall and Perlmann, 1971). Yan and Lv (1978) were the first to develop and use ELISA for the diagnosis of schistosomiasis in The People's Republic of China. The sensitivity of routine applications of ELISA based on SEA reaches 95–99% in *S. japonicum* egg-positive individuals with a conversion rate of 59–60% 1–2 years after effective treatment, while the false positive rates in a nonendemic area remain low at 1–4% (Li, 1991). Special treatment of the antigens or modification of the assay components can improve the diagnostic efficacy of ELISA and extend its application. Various ELISA modifications, including the

Falcon assay screening test (FAST-ELISA), the avidin-biotin-peroxidase complex assay (ABC-ELISA) and the fractioned-antigen (FA-ELISA), have been explored and developed for antibody detection (Wang and Li, 1990; Zhang et al., 1986; Zhu et al., 1996). In the past 20 years, many assays based on recombinant peptide antigens have reported excellent sensitivity and specificity, eg, rSj26GST (Xie et al., 1995), rSj23HD (Ren et al., 2001), rSj32 (Shu et al., 1998). The rSP13-ELISA tool developed by Dr Xu et al. (2014b) have shown substantial advantages over simple egg-detection and SEA-ELISA, including adequate sensitivity and specificity needed in areas characterized by low levels of transmission. Application of such tools may allow identification of cases with low-intensity infections for targeted treatment (Zhou, 2014).

With the advent of the hybridoma technique by Kohler and Milstein (1975), a number of monoclonal antibodies reactive with specific schistosome antigens were developed in The People's Republic of China and used for the detection of CAg (Zhu, 2005). Using nitrocellulose and other paper membranes as carrier, Yan et al. developed a dot-ELISA kit with a monoclonal antibody against the *S. japonicum* circulating cathodic antigen (CCA) linked with peroxidase, which detected 90.6% and 83.2% of acute and chronic schistosomiasis patients, respectively (Yan et al., 1990). Although various ELISA-based monoclonal antibody assays on for antigen detection were explored by Chinese scientists, they are not widely used due to unsatisfactory sensitivity in patients, especially with light infections (Guan and Shi, 1996; Yi et al., 1995). As an ELISA reader is required for most kits and the delay before it is possible to inspect the results is usually 2—3 h, ELISA is mainly a laboratory-based tool that cannot be easily adapted for large-scale use in field settings.

2.3.5 Rapid diagnostic kits

As diagnosis in the field needs to be settled at the point of care, which means minutes rather than waiting a few hours as the case is with ELISA-type assays. To that end, rapid diagnostic kits based on immunofiltration or immunochromatography principles with dyes or colloid metals as markers have been developed. For example, Ding et al. (1998) developed the dot immunogold filtration assay (DIGFA) for schistosomiasis japonica diagnosis based on SEA as antigen and rabbit—antihuman IgG labelled with colloidal gold as probe, while Tang et al. (2008) developed the assay further by using a sheep antihuman IgM immunogold conjugate as probe to detect specific IgM anti-*S. japonicum* antibody that produced positive rates for acute and chronic schistosomiasis at l00% and 96% sensitivity, respectively. The specificity

was also good, with only 3% of patients infected with *Paragonimus westermani* reacting positively in the test (Tang et al., 2008). The dipstick dye immunoassay (DDIA) is another rapid diagnostic approach, which is basically a chromatography technique with SEA labelled with a dye as indicator. It was first produced by He et al. (2000) and further developed by Zhu et al. and Song et al. (Song, et al. 2003; Zhu, et al. 2002; Zhu, 2005). This test has a high sensitivity for both acute and chronic cases of schistosomiasis (97% and 94–97%, respectively) and specificity of 97% when tested with healthy people from nonendemic communities. This assay was also adopted for *Schistosomiasis mekongi* diagnosis (Zhu et al., 2005b), while Ding and Yu modified the technique and made it capable of the detection of specific antibodies also in whole-blood samples, ie, the dipstick latex immunochromatography assay (DLIA). It has high sensitivity (95% for serum and 94% for whole-blood) and specificity (95% for serum and 97% for whole-blood) with no cross-reaction with *Clonorchis* or intestinal nematodes including *Angiostrongylus cantonensis* (Ding et al., 2010; Yu et al., 2011). All the rapid diagnostic assays mentioned previously are suitable for general field screenings since they can be read within 5–10 min without need of a microscope or other special equipment.

It should finally be mentioned that recent advances in the detection of CAg have solved the problem of low sensitivity that has plagued this approach for decades. The upconverting phosphate lateral flow (UCP-LF) (Corstjens et al., 2014; van Dam et al., 2013) presents previously unimaginable sensitivity (a few worm pairs) and specificity (Corstjens et al., 2014). A small-scale field trial conducted in a low-endemic areas in The People's Republic of China has shown that UCP-LF can be used for the diagnosis of *S. japonicum* infection based on the excretion of CCA in urine samples (van Dam et al., 2015), a fact that will aid complacency. The assay exhibited a much higher sensitivity than that of the Kato-Katz technique and detected a significant number of egg-negative cases.

2.4 Techniques based on molecular biotechnology

Better diagnostic tests for schistosomiasis are needed both in the field and in the clinic. Future research for the diagnosis of aetiology may depend on molecular tools such as PCR- or LAMP-based approaches. Improved results have been achieved with multiplex PCR (amplification of several different DNA sequences simultaneously) and real-time PCR (monitoring the amplification of a targeted DNA molecule continuously rather than only at its end) (ten Hove et al., 2008).

PCR has shown great sensitivity and specificity for the detection of *Schistosoma* DNA in a variety of samples (Gomes et al., 2010; Pontes et al., 2003;

ten Hove et al., 2008). After Lier et al. proved the feasibility of molecular methods for detecting *S. japonicum* DNA (Lier et al., 2006, 2008, 2009), Xia et al. developed a specific PCR assay for the highly repetitive retrotransposon SjR2 of *S. japonicum* DNA based on an animal model infected with *S. japonicum* detecting *S. japonicum* DNA down at 0.8 pg (10^{-12} g), which approaches the level of one EPG of stool. *Schistosoma japonicum* DNA could be detected in sera at the first week postinfection and became negative 10 weeks posttreatment (Xia et al., 2009). Fung et al. (2012) developed a faecal PCR assay using the same primer to detect *S. japonicum* infection in humans and bovines in The People's Republic of China. The test was highly sensitive, detecting *S. japonicum* DNA at 0.5 EPG of stool.

Based on the sequence of SjR2, a LAMP assay, without requirement of a thermocycling machine or electrophoresis equipment, was designed and the results showed that this method was able to detect 0.08 fg (10^{-15} g) S. *japonicum* DNA, which was 10^4 times more sensitive than conventional PCR (Xu et al., 2010, 2015). The test has a high sensitivity of 95–97% for the diagnosis of *S. japonicum* infected patients with the lowest intensity (EPG <10) (Xu et al., 2015). After treatment with praziquantel, the negative conversion rate increased from 23.4% to 83.0% at 3 months and 9 months posttreatment according to LAMP, where the seroconversion rate remained at a low level (25.5% by ELISA and 31.9% by IHA) even 9 months after treatment (Xu et al., 2010, 2015).

These studies presented previously demonstrate that PCR and LAMP are effective diagnostic tools for early diagnosis and evaluation of therapy effectiveness for *S. japonicum* infection in schistosomiasis-endemic areas with low-intensity infection. However, the dependence on expensive apparatus and the need for specialized, trained technicians, restrict the widespread applications of PCR for testing under field conditions (Guo et al., 2012; Kato-Hayashi et al., 2015; Sandoval et al., 2006). As LAMP has the potential value for field use, further studies based on real field settings need to be conducted to evaluate their diagnostic efficacy and operational characteristics. However, it should be borne in mind that stool examination may fail depending on the timing of release of parasite eggs in the stool.

3. DIAGNOSTIC APPLICATIONS IN THE NATIONAL SCHISTOSOMIASIS CONTROL PROGRAMME

Since the 1950s when the national campaign against schistosomiasis began in The People's Republic of China, the application and development

of diagnostic techniques have undergone shifts. However, an approach that is useful in practice is almost always a compromise between quality and quantity because the techniques needed for large-scale application must be based on cost-effectiveness, precision, simplicity and stability (Bergquist et al., 2009). The key of the matter is the required continuous adaption of the diagnostic focus to the stage of control. Fig. 1 shows the various stages of a schistosomiasis control programme juxtaposed with the type of diagnostic tools that should be employed to reach the set goals.

3.1 The morbidity control stage

At the early stage after the founding of The People's Republic of China, there were a large number of people affected by schistosomiasis, and hence high infection prevalence and infection intensity were very common, especially during the 1950–1980 period. Treatment of patients with severe illness to save their life and rescue the workforce was the primary task. The etiologic diagnostic techniques, including direct stool smear examination, sedimentation, concentration and hatching, combined with a questionnaire approach, were almost compulsory in the identification of individuals for mass chemotherapy because of high infection intensity and toxicity of available effective drugs, eg, antimonials, for treatment. The parasitological techniques had a better diagnostic efficacy, while the immunodiagnostic approach commonly produced false positive results that increased the number of individuals receiving unnecessary chemotherapy. Eventually, immunodiagnostics were only adapted for screening prior to further parasitological examination. In the national general survey initiated in 1956, the strategy of first screening populations by ID followed by stool examination for those who reacted positively when subjected to ID testing made it possible to rapidly understand the distribution and endemic characteristics of schistosomiasis.

The development and release of praziquantel for clinical use in the 1970s was a milestone for schistosomiasis control (Tambo et al., 2015). High efficacy against all species of human schistosomiasis, use in single oral dose and with none or only mild side effects made praziquantel highly attractive, and when the cost fell dramatically in the 1990s, it could be used for mass drug administration (MDA). Along with the reduced intensity of infection that resulted, the limited sensitivity of the parasitological, diagnostic techniques in use became apparent and control programme managers looked for replacements, such as immunological techniques based on both antigen and antibody detection (Bergquist et al., 2009, 2015; Doenhoff

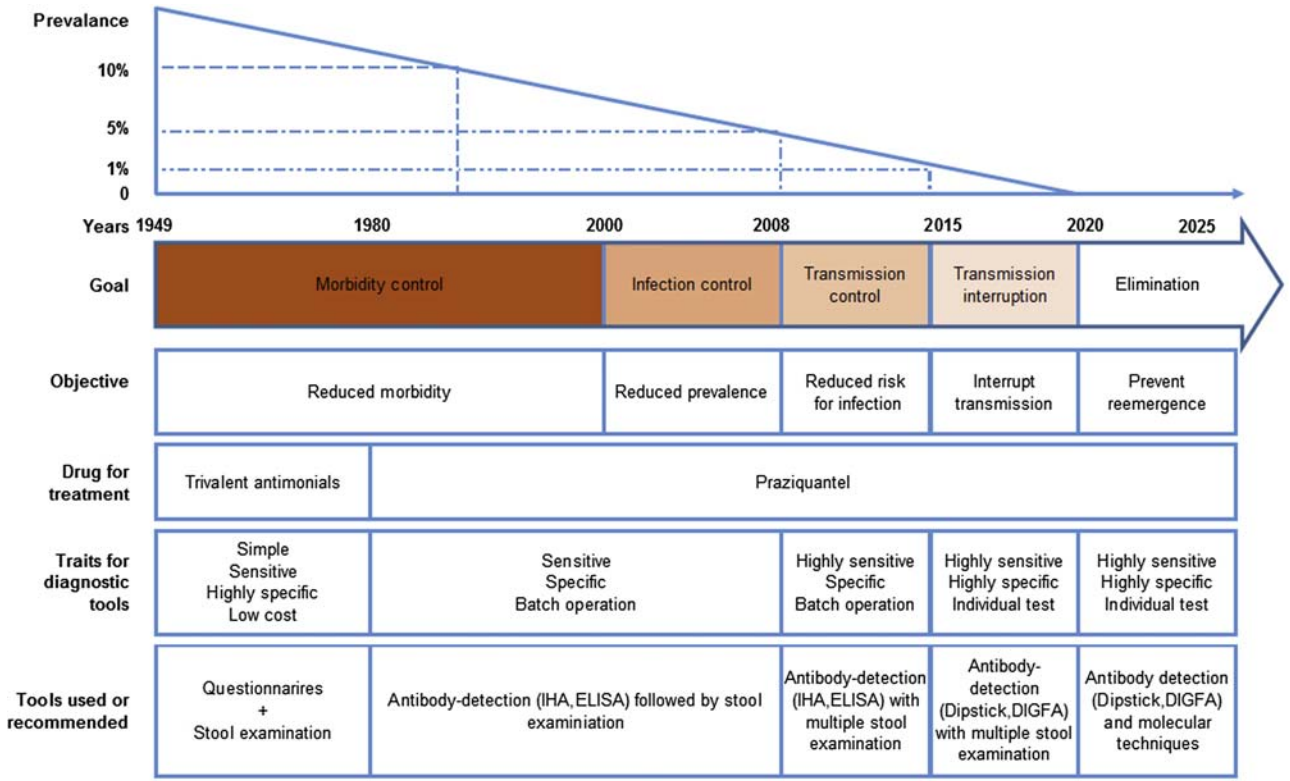

Figure 1 Diagnostic tools shift with the control stage and drug used for schistosomiasis control in The People's Republic of China.

et al., 2004; van Lieshout et al., 2000; Wu, 2002). In the implementation of 10-year World Bank Loan Project (WBLP) for schistosomiasis control initiated in 1992 (Chen et al., 2005) in The People's Republic of China aiming for morbidity control, diagnostic strategies varied according to prevalence strata and chemotherapy strategies. For instance, in areas of high endemicity (prevalence $\geq$15%), all individuals between 6 and 60 years old without examination were given yearly treatment. In areas of medium endemicity (15% > prevalence > 3%) and low endemicity (prevalence <3%), residents or special populations were screened by Kato-Katz method or a serological test (ELISA, COPT or IHA). The final evaluation in 2002 showed that infection rates in humans had decreased by 55% (Chen et al., 2005).

The way used for the epidemiological surveys was performed in differed methodologies according to perceived infection status and diagnostic techniques available. Direct stool examination was the only method to evaluate the prevalence of schistosomiasis in the first national sampling survey in 1989. With the development of immunological techniques and the decrease of infection intensity and prevalence in some endemic areas, direct stool examination was only used in highly endemic areas, while serological survey followed by stool examination were used where morbidity control had been reached. In the national survey conducted in 2004 after the WBLP terminated, prevalence was estimated using ELISA screening prior to stool examination as the endemicity of schistosomiasis had then decreased significantly in most endemic counties (Chen, 2014).

3.2 The infection control stage

Once morbidity is under control, infection control through decreasing prevalence and prevention of reinfection is the next target. This situation, characterized by less MDA and predominantly low-intensity infections, requires more sensitive diagnostic techniques to support the distribution of chemotherapy. In 2004, a medium- and long-term national strategic plan for schistosomiasis control was initiated in The People's Republic of China to face the challenges after the termination of the WBLP. To reach the medium goal for infection control by 2008, diagnostic and chemotherapy strategies were determined according to the prevalence of schistosomiasis based on the situation at the administrative village level. Apart from inquiry examinations conducted in villages with prevalence higher than 10%, serological methods were implemented annually, every 2 years or every 3 years for residents aged 6–65 years in villages with an estimated prevalence of 5–10%, 1–5%, 0–1%, respectively. Once infected snails were detected, serological survey would be conducted no matter the

estimated prevalence of schistosomiasis in the same year (Ministry of Health, 2005; Zhou et al., 2007).

In areas having reached the stage of infection control, the survey tools shifted from direct Kato-Katz for all to initial immunological screening to increase the diagnostic efficiency and compliance rate of residents being examined (Zhu et al., 2009). A system with primary immunodiagnostic screen followed by Kato-Katz tests for the antibody-positive individuals (Balen et al., 2007; Utzinger et al., 2005) was advised. This approach is currently widely used in the Chinese national control programme as well as for community surveys and field studies. In addition, to evaluate the effect of interventions conducted and understand the changes in the endemic areas, the number of national sentinel sites was enlarged from 14 in 6 endemic provinces in 1990 to 80 in 9 endemic provinces and potential prevalent areas in Chongqing city with potential risk of endemics in 2005 in the national surveillance system due to the decrease of prevalence and general improvement of the situation (Cao et al., 2016; Zhu et al., 2011).

3.3 The transmission control stage

With the implementation of integrated control strategy based on interventions to reduce the rate of transmission of *S. japonicum* infection from cattle and humans to snails in the national control programme, the prevalence of schistosomiasis decreased to the lowest level in history and infection control was reached nationwide in 2008 (Hao et al., 2009; Wang et al., 2009). The whole country is now moving forward to transmission control, ie, nationwide prevalence <1% based at the village level (Utzinger et al., 2005; Zhou et al., 2005). At this phase, selective chemotherapy with praziquantel becomes the main strategy due to the significant decrease of infection rate in humans. However, this stage requires considerably more sensitive diagnostic tools to accurately identify infected individuals. At this stage, the Kato-Katz 'missing rate' increases significantly and can reach as high as 80% when the level of prevalence, accompanied by much lower infection intensities, falls to the <1% level (Lin et al., 2008; Zhu et al., 2005a). At egg excretion levels from the host below 100 EPG of stool, it becomes increasingly difficult to unequivocally determine whether or not there is an infection (Feldmeier and Poggensee, 1993). Although multiple Kato-Katz thick smears per sample, or increasing the frequency of stool sample collection, would boost diagnostic sensitivity (Huang et al., 2007; Xu et al., 2011a; Zhu et al., 2005a), this approach could not be used at a large scale due to the workload and increased costs as well as the risk of waning compliancy.

The Chinese criteria of schistosomiasis control and elimination recommend evaluating whether a place has reached the stage of transmission by an immunological assay followed by the hatching test as it is regarded as more sensitive than the Kato-Katz technique. Conducting multiple methods for the same stool sample at the same time would also increase the sensitivity of the diagnostic strategy. This approach has been integrated into the revised national surveillance system since 2011 (Zhang et al., 2012). An analysis shows that the parasitological final positive rates for individuals testing positively serologically have increased from 4.6% by the Kato-Katz method and 5.9% by the hatching technique to 7.1% when these two methods are used in combination (Zhu et al., 2013b).

3.4 The stage of transmission interruption and elimination

The People's Republic of China has now reached the stage when the priority is shifting from traditional monitoring to surveillance and response with the focus on reinfection in areas targeted for elimination (Cao et al., 2016; Xu et al., 2014a; Zhou et al., 2013). The most urgent need at this time is reliable assessment of control efficacy and the determination of target populations for chemotherapy in different areas as well as certification of elimination using sensitive and specific assays. The immunological techniques needed now must be fast, highly sensitive and specific. Various approaches fulfil these requirements, eg, molecular methods such as PCR, real-time PCR and LAMP could be conducted in well-equipment hospitals and laboratories; the latter can even be used in the field (Zhang et al., 2016).

The UCP-LF test (Corstjens et al., 2014; van Dam et al., 2013), on the other hand, detects CAg at a previously unimaginable sensitivity and specificity making it possible to detect infections due to only a few worm pairs (Corstjens et al., 2014). The other great advantage with this approach is that it can detect CAg both in serum and urine, the latter being more acceptable to the general public should allow the large number of test that will be needed in the elimination phase. However, further technical improvements may be needed to make the test more convenient, simple and applicable for large-scale field operations.

4. QUALITY CONTROL

Diagnosis would supply information for planning the control activities as well as assessing the strategy or effects of control measures. As many factors, such as laboratory conditions, diagnostic methods/assays chosen,

technician capacity, etc. impact the quality of the final diagnosis (Tokmalaev and Bezborodov, 1982). The People's Republic of China has strengthened monitoring and management with respect to diagnostic activities. For example, criteria for schistosomiasis diagnosis have been issued to provide guidance for the technical staff and standardize the diagnostic approach. Guidelines are regularly updated by the Ministry of Health with consideration to available technology and supplies as well as the stage of control and clinical symptoms, including history of water contact, previous laboratory examinations (Minstry of Health, 2006). These criteria define the principles and basis for diagnosis and differentiation between acute, chronic and advanced cases.

Assessment of the efficacy, reliability and operational characteristics of assays in use are important for the assurance of the diagnostic accuracy. With the development and implementation of CAg assays in The People's Republic of China, three collaborative studies were conducted (in 1993, 1995 and 1996) to evaluate all existing CAg kits. In 1993, eight assays using monoclonal or polyclonal probes were evaluated and the results showed that most of the tests had low sensitivities ranging from 15% to 73% in chronic and light infection, respectively (Yi et al., 1995). The evaluation conducted in 1995 showed that 10 of 14 kits investigated had a specificity above 90% but only four assays showed a sensitivity higher than 60% (Guan and Shi, 1996). In 1996, 12 CAg kits displayed a similar diagnostic efficacy as in 1995 (Feng et al., 1998). These evaluations demonstrate that the CAg kits evaluated were inappropriate for use by the schistosomiasis control programme at that time, especially in endemic areas characterized by low-level infections. At the same time, nine assays for antibody detection were evaluated and the results showed that most kits had a specificity over 90% and sensitivity higher than 80% in chronic infection (Feng et al., 1998). Comprehensive evaluations conducted in 2004 and 2008, respectively, also showed that the sensitivity and specificity both were above 90% for most reagents (Xu et al., 2005, 2011b). A field evaluation conducted later proved that these antibody-based assays were acceptable as tools for community survey and for targeting chemotherapy.

The results of the assessments mentioned previously demonstrate that many diagnostic methods in use need to be controlled for quality, and when they are investigated the standards are often not good enough. Before 2008, many assays for schistosomiasis diagnosis were used without having passed certification by the State Food and Drug Administration (SFDA) in The People's Republic of China. However, the evaluations carried out

provided data for that the manufacturer could use to apply for licence from SFDA. Until now, nine kits have been accredited by SFDA, which indicates that the situation is improving and already some assays are quality controlled with good results (Table 1).

To strengthening the capacity for schistosomiasis diagnostics, the Chinese Centres for Disease Control (China CDC) organizes since 2009 a diagnostic platform run through a set of reference or sentinel laboratories for external quality control activities, and also holds training courses. So far, one national diagnostic centre has been established in the National Institute of Parasitic Disease, Shanghai, The People's Republic of China. It is responsible for assay assessments; external control and improvement of the diagnostic quality control scheme; planning and organizing training activities; and providing technical support for subordinate laboratories. Further, 12 reference laboratories at the provincial institutes and 16 sentinel laboratories at the county level were set up in charge of the activities related with diagnostic activities (Qin et al., 2013; Zhang et al., 2016). Subsequently, several provinces established their own diagnostic platforms to enhance management of diagnostics. Chen et al. (2011b) organized three external control activities focused on the hatching technique in 2006, 2008 and 2009, respectively. The average accuracy rates of the hatching technique in 18, 28 and 33 laboratories in Zhejiang

Table 1 Immunodiagnostic assay kits for schistosomiasis diagnosis registered and accredited by The People's Republic of China Food and Drug Administration

Type of assay	Probe/target	Company
IHA[a]	SEA/IgG	Yueyang Xunchao Biotechnology Co., Ltd.
IHA[a]	SEA/IgG	Anhui Anji Medical Technology Co., Ltd.
DIGFA[b]	SEA/IgG	Yueyang Xunchao Biotechnology Co., Ltd.
DIGFA[b]	SEA/IgG	Sichuan Maike Biotechnology Co., Ltd.
DIGFA[b]	SEA/IgG	Shanghai Jikong Biotechnology Co., Ltd.
DDIA[c]	SEA/IgG	Wuxi Saide Technology Development Co., Ltd.
MPAIA[d]	SEA/IgG	Beijing Beiaikang Biological technology Co., Ltd.
ELISA[e]	SEA/IgG	Shenzhen Huakang Biomedical Engineering Co., Ltd.
ELISA[e]	Not clear/ Circulating antigen	Sichuan Maike Biotechnology Co., Ltd.

[a]Indirect haemagglutination assay.
[b]Dot immunogold filtration assay.
[c]Dipstick dye immunoassay kit.
[d]Magnetic particle antibody immunoassay.
[e]Enzyme-linked immunosorbent assay.

Province (where transmission was interrupted in 1995) were 88.9%, 100% and 93.9%, respectively. The diagnostic capacity was strengthened through repeated training and control activities.

5. CURRENT CHALLENGES

In the process of schistosomiasis control, rapid and reliable diagnostic techniques are necessary and important to identify targets accurately for treatment. Current diagnostic technology for schistosomiasis is useful in The People's Republic of China but big challenges exist when moving towards elimination.

Firstly, the stability and quality of the diagnostic assays are overestimated. Although most diagnostic kits/assays performed excellent diagnostic efficacy based on laboratory evaluation, or in a small-scale field assessment, their sensitivity and/or specificity normally decreased when evaluated in the field (Xu et al., 2007; Zhou et al., 2011) Although there are several immunological kits accredited by SFDA, the quality of immunological diagnosis kits available in the market is uneven (Xin et al., 2006). Novel approaches, such as PCR and LAMP, demonstrating excellent sensitivity and specificity as reported, mainly focused on laboratory evaluation, still need multicentre assessment both in laboratories and field settings as there are more factors, such as storage, transportation and operation, etc., influences the diagnostic efficacy and stability of the results. Because of the high sensitivity of PCR and LAMP, attention must be paid to the possibility of false positive by potential pollution in the laboratory.

Secondly, the endemic status is underestimated due to the current diagnostic strategy. The currently available antibody-based serological assays cannot distinguish active infection from previous infection or reinfection, which makes it difficult to determine prevalence directly. Theoretically, if the sensitivity of a screening tool is less than 100%, the sensitivity of current diagnostic strategy using serological screening followed by stool examination will be lower than that of direct stool examination, which hinders the process of schistosomiasis elimination due to missed infected cases although they may be very few. Although detection of schistosome CAg is regarded as an effective approach in theory, only the UCP-LF approach has shown the sensitivity required; other assays that have been tested demonstrated an unsatisfactory specificity and insufficient sensitivity, especially in patients with light infections, eg, chronic cases. Further studies should be considered to explore assay systems based on defined antigens or particular antigenic

epitopes and corresponding isotype-restricted antibody responses in combination with the advantages of antibody or antigen detection, which are duration and titre dependent.

Thirdly, the diagnostic capacity in control stations is weak and needs to be strengthened. Feng et al. (2011) conducted a survey showing the poor quality of diagnostic laboratories in charge of national surveillance for schistosomiasis. The results showed that the average age of laboratory staff was 40.93 ± 9.56 years old and 69% of the personnel were older than 35 years, 86% of whom had an education background below the BA degree. Also the equipment in most laboratories needs to be improved and updated. In addition, the diagnostic capacity varied significantly among different places (Wang et al., 2015; Zang et al., 2013). Zhu et al. (2013a) prepared specimen panels with eight common helminth eggs, which was examined by 48 personnel from Hubei and Hunan Provinces. Schistosome eggs were misdiagnosed by 6% of the technicians and negative smears were misdiagnosed by half of them. This results clearly show that the diagnostic capacity must be strengthened, which should include training and modernizing outdated equipment.

6. CONCLUSIONS

Above all, the use of appropriate, sensitive diagnostic tools to identify infected individuals is imperative to control and prevent the morbidity caused by schistosomiasis. Control that appropriate diagnostic tools are applied would be extremely helpful in implementing strategies for the control and elimination of schistosomiasis. The persistence of the disease in some regions where schistosomiasis remains endemic despite massive and integrated control programmes conducted continuously may be partially due to lack of accurate diagnostic tools for case detection and community screening. The most widely used diagnostic approaches in The People's Republic of China are stool examination by Kato-Katz method, the hatching technique, while immunological assays, especially antibody detection, are used for screening. Usually, parasitological tests are performed after a positive screening test to establish a definitive diagnosis.

A variety of diagnostic techniques, ranging from basic microscopic detection to sophisticated molecular approaches, have been developed for detection of schistosome infections. The ideal diagnostic test (or set of tests) should provide an accurate diagnosis preferably performed once, be cost-effective in terms of labour, sample processing, equipment and reagents.

Implementation of appropriate diagnostic tools for all endemic areas and targeted populations remains a determining factor in the acceleration of the progress of the national schistosomiasis control programme in The People's Republic of China. As the next goal is to interrupt the transmission and then finally eliminate schistosomiasis, the test to be used for case detection at this stage needs to have very high sensitivity and specificity as most endemic areas are characterized by low transmission and low prevalence. As the test systems in current use do not meet the stringent criteria that will dominate at the elimination stage, it is imperative to develop more effective approaches for case finding and surveillance.

To ensure the accuracy and reliability of data obtained through diagnostic work, diagnostic capacity building should be strengthened through external quality control and professional training. The quality of the diagnostic tools in use should be monitored based on laboratory or field assessments using the national schistosomiasis laboratory network platforms. As schistosomiasis japonica is a zoonotic disease, this would apply also for the veterinary sciences with respect to the diagnosis of schistosomiasis in livestock (Cao et al., 2016; Yang et al., 2016). These activities will be very important for monitoring the elimination of schistosomiasis.

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