Epidemiology and diagnosis of *Schistosoma japonicum*, other helminth infections and multiparasitism in Yunnan province, People's Republic of China

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Prof. Dr. Hans-Peter Hauri
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To my family

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2. Summary

Background Schistosomiasis is a water-based disease, endemic in over 70 countries in the tropics and subtropics. At present, the bulk of the global burden is concentrated in sub-Saharan Africa. Some important foci exist in South America, the Middle East, Southeast Asia and in China. We reviewed the literature, used the latest population statistics and estimate that globally, 207 million people are infected and 779 millions are at risk of infection. Soil-transmitted helminthiasis caused by *Ascaris lumbricoides*, hookworms and *Trichuris trichiura*, is highly endemic throughout the tropics and elsewhere, particularly in resource-constraint settings. At least 1 billion people worldwide are infected, many of whom harbour multiple species concurrently. *Strongyloides stercoralis* is a far less recognized and researched soil-transmitted helminth. *Taenia* spp. is transmitted via raw and undercooked meat dishes and is endemic globally but, similar to *S. stercoralis*, accurate statistics and distribution maps are lacking. All these parasites belong to the group of the so-called neglected tropical diseases. In recent years, different programmes have been launched with an aim to providing regular anthelminthic treatment to millions of people worldwide. However, only a handful of safe and efficacious drugs are available, but none of them covers the entire parasite spectrum. While chemotherapy is a key strategy to reduce morbidity, other measures are necessary to achieve sustainable control.

Schistosoma japonicum, soil-transmitted helminths, *Taenia* spp. and other helminths are common throughout China. In view of the profound demographic, ecological and socio-economic transformations China has gone through over the past 30 years, the distribution and frequency of many parasites has changed. Shifts have been attributed to regional variations in control efforts, socio-economic development and changing customs.

Objectives This Ph.D. thesis pursued five specific objectives. First, to systematically review the literature with regard to the effects of water resources development on the local epidemiology of schistosomiasis. Second, to study the epidemiology of *S. japonicum* and other helminth infections across Eryuan county, Yunnan province, China. Third, to identify risk factors for *S. japonicum* seropositivity in Eryuan county, and to put forward predictive risk maps. Fourth, to study intestinal multiparasitism, the local endemicity of *S. stercoralis* and the performance of diagnostic tools in Menghai county, Yunnan province. Fifth, to assess the safety and efficacy of tribendimidine against *S. stercoralis* and *Taenia* spp.

Methods The available data regarding the number and spatial extent of large dam reservoirs and surface-irrigation in schistosome-endemic areas were compiled and multiplied with

country-specific rural population density estimates. We systematically reviewed the literature to identify studies pertaining to the effect of water resources development projects on schistosomiasis, and carried out a meta-analysis and calculated risk ratios.

The cross-sectional survey in Eryuan county involved 3220 individuals from 35 villages. They were screened by parasitological (*S. japonicum*, intestinal helminths) and serological methods (schistosomiasis, cysticercosis, trichinellosis). Questionnaires were administered to obtain demographic, behavioural, and socio-economic data. Geographical, remotely-sensed environmental, demographic, and epidemiological data were used in a spatially-explicit Bayesian model to predict the risk of schistosomiasis japonica seroprevalence across the county. The endemic spectrum of intestinal parasites was assessed in Nongyang village in southern Yunnan province where 2-3 stool samples were collected from 215 individuals, and analysed by four different diagnostic approaches, i.e. Kato-Katz, Baermann, Koga agar plate and ether-concentration after conservation of the stool sample in sodium acetate-acetic acid-formaline solution. The effect of the sampling effort on the measured prevalence, and the diagnostic performance of the different techniques were assessed. The safety and efficacy of tribendimidine for treating *S. stercoralis* and *Taenia* spp. infections was investigated in Nanweng village. A single oral dose of tribendimidine was administered to 57 individuals, and results were compared to the effect of a single oral dose of albendazole given to 66 individuals. The efficacy was assessed 2-3 weeks post-treatment based on 2-3 stool samples screened before and after treatment using different methods.

Results A predicted 106 million people at risk of schistosomiasis live adjacent (≤5 km) to large dam reservoirs or in irrigated areas. We identified 58 studies, mainly from African settings, and included a subset of 24 studies containing 35 datasets to calculate pooled random risk ratios. People living in close proximity to large dam reservoirs were at a 2.4-fold (95% confidence interval [CI]: 1.4-3.9) higher risk of *S. haematobium* and at a 2.6-fold (95% CI: 1.4-5.0) risk of *S. mansoni*. In irrigated areas, the pooled random risk ratios were 1.1 (95% CI: 0.02-7.3) for *S. haematobium* and 4.7 (95% CI: 0.49-23.0) for *S. mansoni*.

The most common helminth in Eryuan county was *A. lumbricoides* (15.4%), followed by *Taenia* spp. (3.5%) and *S. japonicum* (2.7%) in known schistosome-endemic villages. Seroprevalences were high; 58.8% for trichinellosis, 49.5% for schistosomiasis japonica in known *S. japonicum*-endemic villages, and 18.5% for cysticercosis. Prevalences as well as the socio-economic status of the families showed strong spatial heterogeneity; most helminths were more prevalent among the poor in mountainous areas but *S. japonicum* and trichinellosis were mainly found among the better-off inhabitants of plain areas. Being Han and growing

tobacco were additional risk factors for *S. japonicum*. Sero- and egg-positive individuals were also found outside the recognized *S. japonicum*-endemic villages. The spatially-explicit Bayesian model identified demographic (age and sex) and geographical (slope and elevation) risk factors, and predicted higher *S. japonicum* seroprevalences for the plain areas when compared to mountainous regions.

Fifteen parasite species were identified in Nongyang village, eight helminths and seven protozoa. The prevalence of the three common soil-transmitted helminth exceeded 85% each. We found a *S. stercoralis* prevalence of 11.7% with a predominance among adult males. The prevalence of intestinal protozoa was lower; the most common was *Blastocystis hominis* (20.0%). Most study participants harboured three intestinal parasites concurrently (range 1-6). Infection intensities were mainly light for *T. trichiura* and hookworm, but moderate for *A. lumbricoides*. The collection of multiple stool samples resulted in higher prevalences, most notably for *S. stercoralis* and hookworms. Pooling results from multiple methods consistently increased the overall sensitivity.

A single oral dose of tribendimidine (200 mg for those 5-14 years old; 400 mg for those ≥15 years old) reduced the *S. stercoralis* prevalence from 19.3% to 8.8% (cure rate: 54.5%, P = 0.107) and the *Taenia* spp. prevalence from 26.3% to 8.8% (cure rate: 66.7%, P = 0.014). Albendazole treatment resulted in comparable prevalence reductions. At treatment evaluation, additional infections were discovered among those previously declared uninfected. These infections were most likely missed before due to lack of diagnostic sensitivity. Considering these "new" infections reduced the net cure rate, most notably for *Taenia* spp. among the albendazole group. For *Taenia* spp., the difference between the tribendimidine and albendazole-specific cure rates became significant (P = 0.001).

Conclusions/significance The distribution map of human helminth infections in Yunnan province, China still has many white spots and important shifts in the spectrum and prevalence of endemic parasites are expected in the face of the ongoing socio-economic development. New survey approaches, diagnostic tools and risk profiling techniques have been introduced, and the local epidemiology of *S. japonicum* and further parasites including helminths and intestinal protozoa has been elucidated. *S. stercoralis* is endemic in Yunnan province. The safety and efficacy of a potentially additional tool in future control efforts for treating *S. stercoralis* and *Taenia* spp. – i.e. tribendimidine – was studied. The studies conducted in the frame of this Ph.D. thesis document the current situation pertaining to various currently neglected parasites and in hitherto unexplored settings, thus providing a base for the articulation of much needed control programmes which respond to local needs.

3. Zusammenfassung

Hintergrund Schistosomiasis ist eine über Wasserkontakt übertragene und in über 70 Ländern der Tropen und Subtropen vorkommende Infektion. Der südlich der Sahara liegende Teil Afrikas trägt die Hauptlast dieser Krankheit. Weitere wichtige Verbreitungsschwerpunkte befinden sich in Südamerika, dem Mittleren Osten, Südostasien und in China. Gestützt auf eine Literaturrecherche und die neuesten Bevölkerungsstatistiken schätzen wir, dass weltweit 207 Millionen infizierte und 779 Millionen einem Infektionsrisiko ausgesetzte Menschen leben. Die durch Bodenkontakt übertragenen Würmer Spulwurm, Hakenwürmer und Peitschenwurm sind in der ganzen Tropenzone und darüber hinaus verbreitet, insbesondere in ärmlichen Gegenden. Mindestens eine Milliarde Menschen sind betroffen, wovon viele mit mehreren Arten gleichzeitig infiziert sind. Weitaus weniger bekannt und erforscht ist der Zwergfadenwurm, ein weiterer durch Bodenkontakt übertragener Parasit. Verschiedene Arten von *Taenia* (Schweine- und Rinderbandwürmer) kommen weltweit vor und werden durch den Verzehr von rohem oder wenig gegartem Fleisch übertragen. Ähnlich der Situation beim Zwergfadenwurm existieren auch zu diesen Parasiten keine verlässlichen Statistiken und Verbreitungskarten. Die erwähnten Parasiten gehören zur Gruppe der sogenannten vernachlässigten Tropenkrankheiten. In jüngster Zeit wurden verschiedene Programme ins Leben gerufen welche das Ziel verfolgen, weltweit Millionen von Menschen regelmässig gegen Würmer zu behandeln. Allerdings sind nur wenige sichere und wirksame Medikamente verfügbar und keines davon deckt das gesamte Parasitenspektrum ab. Die medikamentöse Behandlung verfolgt das Ziel, die Schwere der Erkrankung zu lindern, doch sind weitere Massnahmen nötig, um eine langfristige Kontrolle zu erreichen.

Schistosoma japonicum, durch Bodenkontakt übertragene Würmer, *Taenia* und weitere Würmer sind in China weit verbreitet. Die tiefgreifenden demografischen, ökologischen und sozio-ökonomischen Veränderungen, die China im Verlauf der letzten 30 Jahre erlebte, haben die Verbreitung und Häufigkeit von vielen Parasiten beeinflusst. Diese Verschiebungen können regionalen Unterschieden in den Bereichen der Kontrolle, der sozio-ökonomischen Entwicklung und sich ändernden Gebräuchen zugeschrieben werden.

Ziele Fünf Ziele lagen dieser Dissertation zu Grunde. Erstens, eine systematische Literaturrecherche zum Thema der Auswirkung von Wasserbaumassnahmen auf die lokale Verbreitung von Schistosomiasis durchzuführen. Zweitens, die Verbreitung der Japanischen Schistosomiasis und anderer Wurmerkrankungen im Bezirk Eryuan in der chinesischen Provinz Yunnan zu untersuchen. Drittens, Risikofaktoren für Seropositivität für Japanische

Schistosomiasis im Bezirk Eryuan zu identifizieren, und Risikokarten zu erstellen. Viertens, den intestinalen Multiparasitismus, das lokale Vorkommen des Zwergfadenwurmes und die Zuverlässigkeit von diagnostischen Techniken im Bezirk Menghai zu untersuchen. Fünftens, die Sicherheit und Wirksamkeit von Tribendimidin zur Behandlung von Zwergfadenwurminfektionen und Taeniose zu evaluieren.

Methoden Die verfügbaren Angaben zur Anzahl und räumlichen Ausdehnung von grossen Stauseen und Oberflächenbewässerungssystemen in den Schistosomiasisendemiegebieten wurden zusammengestellt und mit länderspezifischen Schätzungen der ruralen Bevölkerungsdichte multipliziert. Eine systematische Literaturrecherche diente dazu, Studien zum Einfluss von Wasserbaumassnahmen auf Schistosomiasis zu identifizieren, und eine Meta-Analyse der Berichte führte zur Berechnung von Risikoquotienten.

Die Querschnittstudie im Bezirk Eryuan umfasste 3220 Teilnehmer aus 35 Dörfern. Diese wurden mittels parasitologischer Techniken auf *S. japonicum* und intestinale Würmer und mit serologischen Methoden auf Japanische Schistosomiasis, Zystizerkose und Trichinellose untersucht. Demographische, verhaltenspezifische und sozio-ökonomische Daten wurden mit Fragebögen erhoben. Geographische Daten, mittels Fernerkundung ermittelte Umweltindikatoren, sowie demographische und epidemiologische Angaben flossen in ein räumliches Bayes'sches Modell zur Voraussage des Risikos der Seropositivität für Japanische Schistosomiasis im ganzen Bezirk ein. Das Spektrum der lokal vorkommenden Darmparasiten wurde im Dorf Nongyang im Süden der Provinz Yunnan studiert. Dafür wurden 2-3 Stuhlproben von 215 Personen mittels vier verschiedener Diagnostikverfahren untersucht. Bei diesen handelte es sich um die Kato-Katz, die Baermann und die Koga Agarplatten-Technik sowie um die Technik der Etherkonzentration von in Natriumacetat-Essigsäure-Formalinlösung konservierten Stuhlproben. Des weiteren wurden der Einfluss der Untersuchung mehrerer Stuhlproben auf die gemessenen Prävalenzen und die Zuverlässigkeit der diagnostischen Techniken untersucht. Die Sicherheit und Wirksamkeit von Tribendimidin zur Behandlung von Zwergfadenwurminfektionen und Taeniose wurde im Dorf Nanweng studiert. Eine Einzeldosis Tribendimidin wurde 57 Personen oral verabreicht, und die Resultate mit dem Effekt einer 66 Individuen oral verabreichten Einzeldosis Albendazol verglichen. Die Wirksamkeit der Behandlung wurde 2-3 Wochen nach der Medikamenteneinnahme mittels je 2-3 vor und nach der Behandlung gesammelter und mit unterschiedlichen Techniken untersuchter Stuhlproben ermittelt.

Resultate Die Hochrechnung ergab, dass 106 Millionen Menschen sind dem Risiko von Schistosomiasis ausgesetzt und leben in der Nähe (≤5 km) von Stauseen grosser Dämme oder

in bewässerten Gebieten. Wir stiessen auf 58 Studien, v.a. aus Afrika, und davon konnten wir 24 Studien mit 35 Datensätzen zur Berechnung durchschnittlicher Risikoquotienten heranziehen. Menschen, die in der Nähe von Stauseen grosser Dämme leben, sind einem 2.4mal (95% Konfidenzintervall [KI]: 1.4-3.9) grösseren Risiko der Blasenschistosomiasis und einem 2.6mal (95% KI: 1.4-5.0) grösseren Risiko der Darmschistosomiasis ausgesetzt. In bewässerten Gebieten sind die durchschnittlichen Risikoquotienten 1.1 (95% KI: 0.02-7.3) für Blasenschistosomiasis und 4.7 (95% KI: 0.49-23.0) für Darmschistosomiasis.

Der am häufigsten diagnostizierte Wurm im Bezirk Eryuan war der Spulwurm (15.4%), gefolgt von *Taenia* (3.5%) und der *S. japonicum* (2.7%) in schon als endemisch bekannten Dörfern. Die Seroprävalenzen waren hoch: 58.8% für Trichinellose, 49.5% für Japanische Schistosomiasis in schon als endemisch bekannten Dörfern, und 18.5% für Zystizerkose. Sowohl die Prävalenz als auch der sozioökonomische Status der Familien war regional sehr unterschiedlich: Die meisten Würmer waren bei armen Bewohnern der Berggebiete stärker verbreitet während Japanische Schistosomiasis und Trichinellose vor allem bei vergleichsweise wohlhabenden Bewohnern der Ebenen gefunden wurden. Die Zugehörigkeit zur Volksgruppe der Han und das Anpflanzen von Tabak waren weitere Risikofaktoren für Japanische Schistosomiasis. Sero- und eipositive Personen wurden auch ausserhalb der schon bekannten Schistosomiasis-endemischen Dörfer gefunden. Das räumliche Bayes'sche Modell identifizierte demographische (Alter und Geschlecht) und geographische (Hangneigung und Höhe) Risikofaktoren und sagte eine grössere Häufigkeit in Ebenen als in Berggebieten voraus.

Im Dorf Nongyang wurden 15 verschiedene Parasitenarten gefunden, acht Wurmarten und sieben Protozoenarten. Die Häufigkeit jeder der drei gewöhnlichen durch Bodenkontakt übertragenen Würmer überstieg 85.0%. Wir fanden Zwergfadenwürmer mit einer Häufigkeit von 11.7%, v.a. bei erwachsenen Männern. Die Prävalenz von Darmprotozoen war tiefer, mit 20.0% am häufigsten fanden wir *Blastocystis hominis*. Die meisten Studienteilnehmer waren von drei Parasiten gleichzeitig befallen (Spannweite 1-6). Die Infektionsintensität war für Peitschen- und Hakenwürmer zumeist gering und für Spulwürmer mittel. Das Sammeln mehrere Stuhlproben resultierte in höheren gemessenen Prävalenzen, besonders für Zwergfaden- und Hakenwürmer. Die Kombination der Resultate verschiedener Diagnostikmethoden führte durchweg zu einer höheren Gesamtsensitivität.

Eine einzelne orale Dosis Tribendimidin (200 mg für 5-14jährige; 400 mg für ≥15jährige) reduzierte die Zwergfadenwurmprävalenz von 19.3% auf 8.8% (Heilungsrate 54.5%, P = 0.107) und die *Taenia*prävalenz von 26.3% auf 8.8% (Heilungsrate 66.7%, P = 0.014).

Die Behandlung mit Albendazol resultierte in vergleichbaren Häufigkeitsreduktionen. Während der Evaluation der Behandlung wurden weitere Infektionen unter den bisher als nicht-infiziert deklarierten Personen entdeckt. Diese Infektionen wurden früher höchstwahrscheinlich aufgrund der mangelnden diagnostischen Empfindlichkeit übersehen. Der Miteinbezug dieser „neuen“ Infektionen reduzierte die Nettoheilungsrate, insbesondere für Taeniose in der mit Albendazol behandelten Gruppe. Im Falle von *Taenia* wurde die Differenz zwischen den Heilungsraten in der Tribendimidin- und der Albendazolgruppe signifikant (P = 0.001).

Schlussfolgerung/Bedeutung Die Verbreitungskarte der menschlichen Wurminfektionen in der chinesischen Provinz Yunnan weist noch immer viele weisse Flecken auf und es wird erwartet, dass sich das Spektrum und die Häufigkeit der einheimischen Parasiten im Zuge der anhaltenden sozio-ökonomischen Entwicklung verändern werden. Neue Erfassungsmethoden, diagnostische Werkzeuge und Techniken zur Risikobeurteilung wurden eingeführt, und die lokale Epidemiologie der Japanischen Schistosomiasis und weiterer Parasiten inklusive Würmer und Darmprotozoen wurde erforscht. Zwergfadenwürmer sind in der Provinz Yunnan heimisch. Die Sicherheit und Wirksamkeit von Tribendimidin, eines weiteren eventuell in der Zukunft zur Kontrolle von Zwergfadenwurm- und Taeniose einsetzbaren Medikamentes, wurden untersucht. Die im Rahmen dieser Dissertation durchgeführten Studien dokumentieren die Verbreitung verschiedener gegenwärtig vernachlässigter Parasiten und die Situation in bisher unerforschten Gebieten und stellen so eine Basis zur Formulierung dringend benötigter, auf die lokalen Bedingungen abgestimmter Kontrollprogramme bereit.

4. Introduction

This Ph.D. thesis deals with common human helminths found in Yunnan province of the People's Republic of China (henceforth: China), specifically with schistosomes and intestinal worms. Additional helminth species with extra-intestinal location as well as intestinal protozoa were included in the investigations as appropriate. Therefore, the focus of this introduction is on schistosomes and intestinal, mainly soil-transmitted helminths, while other parasites with intestinal and extra-intestinal localization are only mentioned in quite general terms.

4.1 Schistosomes and soil-transmitted helminths including *Strongyloides stercoralis*: their biology and life cycle

4.1.1 Schistosomes

Human schistosomiasis results from an infection by trematode blood flukes of the genus *Schistosoma*. The major species are *S. haematobium*, the causal agent of urinary schistosomiasis, and *S. mansoni* and *S. japonicum* which provoke the intestinal, hepatic (*S. mansoni*) or hepatosplenic (*S. japonicum*) form of the disease. *S. intercalatum* and *S. mekongi* are further species of regional importance (Gryseels *et al.* 2006). The lifecycle of the mentioned schistosomes includes the human and, in the case of *S. japonicum* also animal, end host and different intermediate host snails. Amphibious *Oncomelania* snails are the only intermediate hosts of *S. japonicum* while *S. mansoni* and *S. haematobium* rely on the aquatic *Biomphalaria* and *Bulinus* snails, respectively (Jordan *et al.* 1993; Utzinger and Keiser 2004). Unlike the other schistosomes, *S. japonicum* is a true zoonotic parasite infecting not only humans but also more than 40 mammalian species. They are important reservoir hosts (Ross *et al.* 1997; 2001; Williams *et al.* 2002; Wang *et al.* 2005b).

The blood-dwelling schistosome fluke pairs constantly release eggs, roughly half of which reach the environment via faecal (*S. mansoni* and *S. japonicum*) or urinary (*S. haematobium*) excretion (Gryseels *et al.* 2006). Excreted eggs only hatch in water. The photo- and chemotactic miracidia infect suitable intermediate host snails where they multiply. The resulting cercariae are mainly released during daytime. They actively target potential end hosts. Upon contact, they penetrate the skin, reach the liver via the blood stream and develop into schistosomulae which migrate further to reach their final peri-intestinal or peri-vesical location (Gryseels *et al.* 2006). Human infection usually takes place during occupational,

recreational or domestic activities involving direct contact with infested water bodies (Jordan *et al.* 1993).

4.1.2 Soil-transmitted helminths

The term 'soil-transmitted helminths' groups various nematodes together that infect humans and share a common source of infection - soil contaminated by faecal matters. The main species are *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) (WHO 2002; Utzinger and Keiser 2004; Bethony *et al.* 2006). The adult worms share a common location - the intestinal tract - and their numerous eggs reach the environment via the faeces but they differ in their modes of transmission and infection. *A. lumbricoides* and *T. trichiura* are transmitted orally by ingestion of mature eggs. After hatching, *T. trichiura* larvae directly reach the colon and mature while *A. lumbricoides* larvae leave the intestinal tract, migrate through various organs including the lung, ascend the trachea, are swallowed and again reach the gastro-intestinal system where they develop into adult worms. Hookworm eggs already hatch in the soil and third-stage larvae (L_3) penetrate the human skin and reach the blood circulation. After a migration that resembles the way taken by *A. lumbricoides* larvae they also settle and mature in the intestinal tract (Bethony et al. 2006).

Strongyloides stercoralis is another soil-transmitted helminth that transcutaneously infects humans and reaches its intestinal habitat after a migration that includes the bloodstream, lungs, trachea and oesophagus. However, this parasite differs from other intestinal helminths inasmuch as the larvae already hatch in the intestinal lumen where infective stages can develop, enabling autoinfection and, therewith, indefinite persistence of infection (Keiser and Nutman 2004).

4.1.3 Taeniasis/cysticercosis

Taeniasis or tapeworm disease results from the ingestion of raw or undercooked beef (*Taenia saginata*) or pork (*T. solium, T. asiatica*) meat containing cysticerci. These larval stages then develop in the human intestine and after maturation, the adult tapeworms release proglottids containing eggs which are taken up again by cattle or pigs where the larvae hatch in the intestinal tract and migrate to other locations, especially muscles (Craig and Ito 2007). Humans can also act as an intermediate host for *T. solium*, the causal agent of cysticercosis.

Ingested eggs hatch and the oncospheres reach their target tissues via the bloodstream. Most cysticerci develop in subcutaneous tissues or muscles but neural or ocular localization is also possible (García *et al.* 2003). No prevalence or burden estimates of taeniasis or cysticercosis are available (García *et al.* 2003; Hotez *et al.* 2006).

4.1.4 Intestinal protozoa

Various protozoa can colonize the human intestinal system. They share the direct transmission route, i.e. faeco-oral, and usually cause diarrhoea and other intestinal disease. Invasive tendencies and liver abscess formation can be seen in people infected with *Entamoeba histolytica*. Otherwise, intestinal protozoa are a diverse array of organisms. Some of them are pathogenic (e.g. *Giardia intestinalis*, *Entamoeba histolytica*, *Balantidium coli* and members of the *Coccidia* and *Microspora* families) while the pathogenic potential of others is debated (*Blastocystis hominis*, *Dientamoeba fragilis*). Additional amoeba and flagellates are harmless commensals (Farthing 2006).

4.2 Environment and its impact on the endemicity of human parasites

Environmental conditions govern the potential distribution of a range of parasites with free-living or vector-dependent stages. The main determinants are the climate (temperature, precipitation), the geographical conditions including soil-type, slope, wetness and water bodies, and the vegetation. Most of these factors are interrelated, acting upon each other in multiple ways and in complex causal webs (Patz *et al.* 2000). In China, a nationwide correlation between the prevalence of soil-transmitted helminths and the annual average temperature, the extreme lowest temperature, annual relative humidity, sunshine and precipitation was observed when data from the first national sampling survey were analyzed (Xu *et al.* 1995).

Humans are subject to the local conditions and adapt to the prevailing environment, most notably in traditional and rural societies. But they also act upon the environment, thereby influencing the local endemicity of parasites. Environmental habitats as well as hosts and vectors of parasites can disappear as a result of human action and ways of transmission can be blocked due to behavioural adaptations. Human activities also create new habitats and changes in behaviour open additional ways of transmission (Patz *et al.* 2000). Prominent examples include the re-emergence of schistosomiasis japonica in Sichuan which has been attributed to shifts in local socio-economic, environmental and control conditions (Liang *et al.*

2006) and the emergence of food-borne trematodiasis in the wake of altered food consumption patterns (Keiser and Utzinger 2005).

The alteration of local water bodies for irrigation, drainage, power generation or flood protection is one of the most common and influential environmental impacts of prolonged human activity in an area. Done since ancient times and in all parts of the world, water resources development and management often exerts profound positive or negative influence on the local epidemiology of different infectious diseases, be they water-based like schistosomiasis (Grosse 1993; Hunter *et al.* 1993; N'Goran *et al.* 1997) or water-related vector-borne like malaria, lymphatic filariasis, onchocerciasis and Japanese encephalitis (Jobin 1999; Erlanger *et al.* 2005; Keiser *et al.* 2005a; 2005b).

Local environmental conditions can be directly measured on the spot but the ground-based detailed assessment at regional or national level is time-consuming and expensive. Remote sensing (RS) offers a convenient tool to assess different environmental parameters over extended areas. After integration into a geographical information system (GIS), the acquired data can be used to identify zones where the ecological conditions are suitable for parasite occurrence (Beck *et al.* 2000; Goetz *et al.* 2000; Hay 2000; Bavia *et al.* 2001; Bergquist 2001; Brooker 2002; Brooker *et al.* 2006b; Rinaldi *et al.* 2006; Brooker and Utzinger 2007). GIS and RS are especially powerful if combined with further demographic, socio-economic and parasitological data acquired through traditional ground-based means. This approach even allows the identification of population strata at high risk of infection or disease for conditions with focal transmission and occurrence, as it is often seen in parasitic diseases like schistosomiasis and other helminth infections (Raso *et al.* 2005; 2006; Clements *et al.* 2006). Risk prediction has to take into account the scale and aim of investigation as environmental factors govern the potential endemicity of parasites in a specific area but socio-economic and demographic determinants often take prominence and heavily influence the actual situation at local scale (Liang *et al.* 2007).

However, the full potential of GIS and RS can only be tapped in combination with spatial statistics. The development of spatially-explicit Bayesian methods and Markov chain Monte Carlo (MCMC) inference (Basáñez *et al.* 2004) has opened the door for detailed risk profiling. The opportunities offered by the integration of GIS, RS, additional data, and powerful statistical methods have been acknowledged (Gemperli *et al.* 2004; Raso *et al.* 2006; Brooker and Utzinger 2007) and first promising results have been obtained (Zhou *et al.* 2001; Yang *et al.* 2005a; Clements *et al.* 2006; Raso *et al.* 2006; Beck-Wörner *et al.* 2007).

4.3 Global epidemiology of schistosomiasis and intestinal parasites, situation in China and recent trends

4.3.1 Schistosomiasis

Schistosomiasis occurs in Africa, Asia and the Americas. Africa and the Middle East are endemic for *S. haematobium* and *S. mansoni.* Centuries ago, the latter has also been introduced in the Americas. *S. japonicum* has a patchy distribution in China, the Philippines and Indonesia. *S. intercalatum* and *S. mekongi* are confined to Africa and the lower Mekong river in Laos and Cambodia, respectively (Gryseels *et al.* 2006). Globally, more than 200 million people are infected with schistosomes and an estimated 779 million are at risk of infection, predominantly in sub-Saharan Africa (Steinmann *et al.* 2006). Transmission of schistosomiasis was markedly reduced or has ceased in several non- and North African countries but is almost uncontrolled in many sub-Saharan states (Steinmann et al. 2006).

4.3.2 Soil-transmitted helminths

Intestinal helminthiasis is found throughout the tropics and subtropics with a focus in rural and poor urban populations. Current estimates of the total number of people infected with soil-transmitted helminths stand at 807-1,221 millions for *A. lumbricoides*, 604-795 millions for *T. trichiura* and 576-740 millions for the hookworms (de Silva *et al.* 2003; Bethony *et al.* 2006). While the global number of people infected with soil-transmitted helminths remained remarkably constant (Chan 1997), the distribution has changed over recent decades. The prevalence in China, South-East Asia and the Americas declined considerably while it remained stable in Africa where the marked population growth lead to higher absolute case numbers (de Silva et al. 2003).

S. stercoralis is endemic in tropical and temperate climate zones alike but no precise data is currently available on its global burden and geographical distribution. This lack of data was already noted by Stoll when he published his famous account of the global helminthiasis situation in 1947 and he already suspected gross underreporting (Stoll 1947). The situation has not fundamentally changed ever since. It is currently estimated that 30-100 million people are infected with this parasite but the numbers are acknowledged to only represent rough estimates (Keiser and Nutman 2004; Bethony *et al.* 2006; Vadlamudi *et al.* 2006).

4.3.3 Epidemiology of human helminth infections in China

Human parasitic infections are widespread and diverse in China, both with regard to prevalence rates and species present. Mao (1991) published a list of all parasite species identified among humans in China which mentions, among others, 30 species of protozoa, 12 species of cestodes, 26 species of trematodes and 23 species of nematodes.

4.3.4 Schistosomiasis japonica in China

The *S. japonicum*-endemic are in China can be stratified into three distinct geographical and ecological zones, namely (i) plain regions, (ii) swamp and lake regions, and (iii) hilly and mountainous regions (Chen and Feng 1999). In the 1950s, an estimated 11.6 million Chinese were infected with schistosomes (Utzinger *et al.* 2005; Zhou *et al.* 2005). By this time, a dedicated national agency for schistosomiasis control with branches in the major endemic counties was set up and large-scale control activities were initiated. Both enjoyed steady support from the central government ever since (Bundy and Gottlieb 1999; Utzinger *et al.* 2005). From their outset, control activities followed an integrated approach, with strong emphasis on snail control by mollusciciding and environmental modification, and morbidity control through large-scale chemotherapy campaigns. Health education supplemented these activities (Chen 2002). Additional momentum for the control of schistosomiasis in China came through a World Bank Loan Project that was implemented from 1992-2001 (Chen and Feng 1999; Chen *et al.* 2005). According to the results of the "third nationwide cluster sampling survey on the epidemiology of schistosomiasis in the People's Republic of China" carried out in 2004, there are currently 726,000 cases of schistosomiasis japonica in China (Zhou *et al.* 2007), down from approximately 1.5-1.6 million in 1989 (Ross *et al.* 2001; Chen *et al.* 2005; Zhou *et al.* 2005).

Mass-screening and treatment campaigns are run at regular intervals in villages at risk of schistosomiasis japonica transmission, i.e. where infected snails are present or suspected. The frequency of screening and drug administration depend on prevalence levels (Chen and Feng 1999). Nowadays, screening usually relies on serological testing by a sensitive but less specific method and subsequent parasitological confirmation in those found positive (Wu 2002; Xiao *et al.* 2005b; Zhu 2005). Mass-screening is increasingly being replaced by targeted screening and treatment of high-risk groups, e.g. fishermen (Tang *et al.* 2001; Guo *et al.* 2005).

Control activities cover all schistosome-endemic areas and local elimination was achieved in 5 out of 12 endemic provinces and 323 counties, mainly in plain and coastal hilly regions (Chen and Feng 1999; Zhou et al. 2005). However, *S. japonicum* is still endemic in 110 counties and an estimated 15 million people reside in endemic villages (Zhou et al. 2007). Recent data suggest that intermediate host snail habitats are expanding again (Zhang and Wong 2003; Liang *et al.* 2006; Zhou *et al.* 2007). Re-emergence could be associated with a recent predominance of chemotherapy in the set of employed control tools. It has been acknowledged that chemotherapy-only control programmes are not sustainable, since prevalence rates rise again shortly after phasing out of such programmes (Gong and Yang 1999; Zhang and Lin 2002; Utzinger *et al.* 2003; Bergquist *et al.* 2005; King *et al.* 2006; Singer and de Castro 2007).

A distinct epidemiology of schistosomiasis japonica has been noted in the mountainous areas of Sichuan and Yunnan provinces where the mean infection intensity is usually lower than in other endemic areas (Yuan 1995). However, there is a dearth of studies on risk factors for *S. japonicum* and other parasitic infections in Chinese mountainous areas published in the English literature (Spear *et al.* 2004). The local intermediate host snail, i.e. *O. hupensis robertsoni,* is a genetically distinct *Oncomelania* subspecies (Ross et al. 2001) that only occurs at higher elevations than 500-1000 m above sea level. It has a patchy distribution in upstream portions of watercourses (Yuan *et al.* 2002) but is more evenly distributed downstream (Yuan 1995). Infected snails are usually found close to residential areas and on pastures (Yuan 1995; Li and Gong 2003), indicating an important role of livestock in the local epidemiology (Dai *et al.* 1991; Dai and Yan 1993; Zhao and Yi 2000). It was found that livestock represent the most important reservoir and source of infection in valleys whereas humans are the main reservoir in basins (Yuan 1995; Spear et al. 2004). A positive association between the mean prevalence in humans and the development of the livestock population was also noted (Zheng *et al.* 1997). The prevalence in people living along the same stream is often inversely associated with elevation (Yuan 1995; Zheng 2000). Further studies identified an association between the main crop type and the village-level prevalence: manure-based fertilizer was mainly used to grow vegetables and tobacco while rice fields received chemical fertilizer (Spear et al. 2004). Socio-economic risk factors were also identified (Zheng *et al.* 1995; 1996).

4.3.5 Common soil-transmitted helminths in China

Traditionally, China is believed to accommodate about half of the global number of soil-transmitted helminthiasis cases (Stoll 1947) and it probably still does (de Silva et al. 2003).

According to the first national sampling survey of human parasitic infections in China, carried out between 1988 and 1992 and involving the screening of approximately 1.48 million Chinese, the overall prevalence of *A. lumbricoides* was 47.0%, that of *T. trichiura* 18.8% and that of hookworms 17.2% (Yu *et al.* 1994; Xu *et al.* 1995). With regard to hookworm infections, both *A. duodenale* and *N. americanus* were present (Xu et al. 1995). Within the country, a tendency for higher prevalences in humid and tropical areas and an increase from North to South and from West to East were observed (Xu et al. 1995). At the province level, the prevalence rates were significantly associated with the paddy field area per capita of the respective province (Lai and Hsi 1996). Data derived from the second national sampling survey implemented 2001-2004 indicate significant changes in the epidemiology of soil-transmitted helminthiasis; the respective prevalence rates dropped to 12.7%, 4.6% and 6.1% with the highest prevalences now found in western and central provinces (Ministry of Health 2005). The south-western province of Yunnan lags behind the economic development of eastern China and the prevalence reduction over the recent decade was less pronounced. The province-wide prevalence of soil-transmitted helminth infections at the time of the second national sampling survey was 21.7%.

4.3.6 S. stercoralis*, intestinal protozoa and food-borne helminth infections in China*

There is a paucity of epidemiological data on the distribution of *S. stercoralis* and intestinal protozoa in China. These parasites were investigated during the first national sampling survey on human parasitic infections and very low prevalences were reported. However, the employed diagnostic methods have substandard sensitivity (Yu et al. 1994; Xu et al. 1995) and several-fold higher prevalences were found in southeast Asian countries, namely Thailand and Laos (Kasuya *et al.* 1989; Vannachone *et al.* 1998; Nontasut *et al.* 2005).

Food-borne trematodes, e.g. *Clonorchis sinensis* and *Paragonimus* spp. and other food-borne helminthic infections like taeniasis, cysticercosis and trichinellosis are commonly found in distinct regions across China (Yu *et al.* 1994; Li *et al.* 2001; Liu and Boireau 2002; Ito *et al.* 2003; Chen *et al.* 2004; Lun *et al.* 2005; Ministry of Health 2005; Wang *et al.* 2006b). Their endemicity is usually associated with specific ethnic groups or dietary habits. Current estimates of the number of cases often exceed previous ones, e.g. for *Trichinella* spp.,

Paragonimus spp. and *C. sinensis* (Hotez *et al.* 1997; Liu and Boireau 2002; Keiser and Utzinger 2005; Ministry of Health 2005; Cui *et al.* 2006). The estimated number of cases of the latter has tripled to 15 million over the past 10 years (Lun *et al.* 2005). This increase might be due to shifts in food habits, increased long-distance transportation and trade of foodstuff, and inadequate food control and safety inspection (Li *et al.* 2001; Ministry of Health 2005). Additional parasites such as *Angiostrongylus cantonensis* are still rare but the number of cases is rapidly expanding (Lv *et al.* 2007).

4.3.7 Recent public health trends in China

Systematic infection and morbidity control programmes based on the large-scale administration of safe and efficacious anthelmintic drugs like albendazole and mebendazole is a key factor for the observed decline of soil-transmitted helminthiasis prevalences (de Silva et al. 2003; Utzinger and Keiser 2004). Equally important or even more decisive is the unprecedented socio-economic development of China which started in the eastern coastal provinces and is rapidly spreading west. Improved economic perspectives have profoundly affected the living conditions of hundreds of millions of urban and increasingly rural Chinese. These changes also exert a dramatic impact on the public health situation in the country (Banister and Zhang 2005; Huang and Manderson 2005; Ministry of Health 2005). Today, the better-off, mainly urban population of China is faced with the typical health burden of developed countries with a preponderance of non-communicable, chronic diseases (Shi *et al.* 2005; Wang *et al.* 2005a; Liu 2007). Meanwhile, the common infectious and parasitic diseases of the poor are still endemic in areas that are lagging behind in their economic development (de Silva et al. 2003). In these areas, people are increasingly faced with a double burden – firstly that exerted by the traditional infectious diseases and secondly an additional burden stemming from non-communicable conditions related to smoking, alcohol abuse, air pollution and new diets, among others (Utzinger and Weiss 2007). In addition, new infectious diseases including various helminths and HIV/AIDS emerge or spread in the wake of changed dietary and general behavioural habits (Hotez et al. 1997; Lun et al. 2005; Shao 2006; Anonymous 2007; Lv et al. 2007). The market-oriented reform of the health sector has also created new health risks for poor and marginalized population segments who lack or increasingly loose access to adequate medical services (Bian *et al.* 2004; World Bank 2005).

This epidemiological transition, i.e. the replacement of infectious diseases by chronic non-communicable conditions as the main source of local disease burden, is observed

throughout the world in societies experiencing improved socio-economic conditions (Kanavos 2006; Lopez and Mathers 2006). Of particular interest for the situation in China is the experience from Japan and Korea where a striking negative correlation between the gross national product and the prevalence of soil-transmitted helminths has been noted (Hong *et al.* 2006; Ohta and Waikagul 2006). The scheme for the successful control of parasitic infections in Japan has also been promoted in other countries (Kobayashi *et al.* 2006).

4.4 Morbidity and public health effects due to schistosomiasis and STH infections

4.4.1 Schistosomiasis

Schistosomiasis-related morbidity has two manifestations. First, an acute hypersensitivity reaction against migrating schistosomula also known as Katayama fever (Ross *et al.* 2007) and second, chronic disease resulting from the presence of schistosome eggs that fail to reach the intestinal or bladder lumen and get trapped in the peri-intestinal or peri-vesical tissues. Eggs can also migrate to more distant parts of the human body. Schistosome eggs release different proteolytic enzymes provoking inflammatory or granulomatous reactions. Later, they are calcified or embedded into fibrotic tissue. *S. haematobium* eggs trapped in the vesical or urethral walls cause local ulceration and pseudopolyposis resulting in haematuria, a diagnostic finding in urinary schistosomiasis (Lengeler *et al.* 2002; Gryseels *et al.* 2006). Fibrotic reactions lead to hydronephrosis and kidney failure. Squamous bladder cancer has also been linked to urinary schistosomiasis. Intestinal schistosomiasis is caused by the other schistosome species. It is characterised by intestinal bleeding from microulcers and often bloody diarrhoea. Chronic disease due to *S. mansoni* and Asian schistosomes is associated with extensive liver pathology resulting from periportal fibrosis. Life-threatening bleeding from gastro-oesophageal varices is seen in severe cases (Gryseels et al. 2006).

4.4.2 Soil-transmitted helminthiasis

Light and moderate soil-transmitted helminth infections are often associated with little or no acute disease whereas heavy infections can lead to life-threatening conditions like intestinal obstruction (*A. lumbricoides*), acute dysentery (*T. trichiura*) and severe blood loss and anemia (hookworm). Of particular public health significance are the chronic impairments resulting from untreated infections of any intensity. However, these chronic and unspecific health effects are often difficult to measure and their association with a particular parasite is even more challenging (Keusch and Migasena 1982). Since *A. lumbricoides* and *T. trichiura*

prevalences and infection intensities peak among children and young adults whereas uncontrolled hookworm prevalences remain high throughout adulthood, most effects resulting from infections with the former two parasites are seen among children while hookworm-related morbidity is also found in adults, particularly women of child-bearing age (Hotez *et al.* 2006).

Chronic ascariasis leads to reduced vitamin A absorption and lactose intolerance and the constant blood loss resulting from hookworm infection gives rise to iron-deficiency anemia and protein malnutrition (Bethony et al. 2006). Together, these symptoms lead to nutritional deficits which manifest themselves in impaired physical growth and fitness including worker productivity and impact on cognition, school attendance and –performance (WHO 2002; Miguel and Kremer 2004; Ezeamama *et al.* 2005a; Bethony *et al.* 2006). Anemia has been identified as one of the major public health problems associated with neglected tropical diseases (Hotez et al. 2006). Of particular concern is the effect of anemia among pregnant women on the course and outcome of pregnancy (Bethony et al. 2006). The functional significance of even low-intensity multiple helminth infections on clinical outcomes (e.g. anaemia) is increasingly appreciated (Ezeamama et al. 2005a).

S. stercoralis infections in healthy, immuno-competent humans are usually asymptomatic albeit heavy intestinal infections can lead to topical symptoms (e.g. enteritids and colitids). Life-threatening systemic hyperinfection occurs in immuno-compromised (e.g. systemic corticosteroids, HTLV-1, organ transplant recipients) or otherwise debilitated patients (e.g. malnutrition and lymphoma) (Carvalho and da Fonseca Porto 2004; Concha *et al.* 2005; Vadlamudi *et al.* 2006).

Multiple species infections are common among disadvantaged population segments, especially in tropical climate zones (Petney and Andrews 1998; Drake and Bundy 2001). Albeit this has already been noted decades ago (Stoll 1947; Buck *et al.* 1978) and confirmed for all tropical continents, namely Africa (Brooker *et al.* 2000; Thiong'o *et al.* 2001; Keiser *et al.* 2002; Tchuem Tchuenté *et al.* 2003; Raso *et al.* 2004), the Americas (Brooker *et al.* 2006a) and Asia (Giboda *et al.* 1991; Needham *et al.* 1998; Waikagul *et al.* 2002), including China (Yu *et al.* 1994; Booth *et al.* 1996), multiparasitism is still routinely ignored in most surveys (Cox 2001) and burden estimates (Brooker and Utzinger 2007). Recent studies have highlighted the public health importance of multiple species infections (Drake and Bundy 2001; de Silva 2003) and it has been demonstrated that concurrent light infections with several parasites can result in serious health effects (Ezeamama *et al.* 2005b).

4.5 Diagnostic techniques for intestinal parasites

4.5.1 General features

A multitude of techniques for the diagnosis of intestinal parasites has been developed over the past century. They can be divided into two broad categories, tools for direct and those for indirect detection of parasites, namely parasitological and immunological methods. The first class directly visualizes parasites or their eggs, either direct or by molecular biological methods, e.g. polymerase chain reaction (PCR) or specific labelled antibodies. The second class relies on the detection of parasite antigens or human antibodies developed in response to the presence of a particular parasite. Classical parasitological methods comprise the Kato-Katz thick smear technique, direct wet smears with or without previous staining and permanently stained smears, concentration methods based on fresh or conserved stool samples (Katz *et al.* 1972; Marti and Escher 1990; García 2007) and more specialized techniques like the Baermann test, the Koga agar plate test, the charcoal and other culture methods, the scotch test (Koga *et al.* 1991; García 2007) and PCR-based diagnostic tests (Pontes *et al.* 2003; Mathis and Deplazes 2006), among others. Indirect methods mainly rely on antigen-antibody reactions in tissues (e.g. skin) and biofluids like serum and stool and include ELISA, IFA, IHA and intradermal tests (Schuster and Chiodini 2001; Zhu 2005).

Both approaches have their strengths and weaknesses. The specificity (and therefore, also the positive predictive value) of direct detection methods approaches 100%, thus there are usually no false-positives. The sensitivity (and the negative predictive value), however, can be low, depending on the density and distribution of parasites or their eggs in the stool sample, the frequency of parasite expulsion (Nielsen and Mojon 1987; de Vlas and Gryseels 1992; Marti and Koella 1993; Sato *et al.* 1995; Engels *et al.* 1996; Yu *et al.* 1998; Utzinger *et al.* 2001; Booth *et al.* 2003) and time delays between sample production and analysis (Dacombe *et al.* 2007). The main remedy to increase the sensitivity of parasitological methods is to employ several diagnostic techniques concurrently and/or to collect and screen multiple stool samples (van Gool *et al.* 2003). Another draw-back of parasitological methods is their inability to detect recent infections, namely during the prepatent period. The reliability of indirect methods is often impaired by insufficient sensitivity and false-positive diagnoses resulting from cross-reactions with non-specific antigens or antibodies. Another problem associated with antibody-based methods is their failure to distinguish active from past infections.

The sensitivity of most diagnostic techniques tends to follow the intensity of infection (Engels *et al.* 1996; Yu *et al.* 1998; Utzinger *et al.* 2001; Booth *et al.* 2003). Thus, the rate of false-negative results is often higher for low-intensity than for heavy infections. Since chemotherapy does not always completely cure infections but rather reduces the number of parasites (WHO 2002), the sensitivity of a diagnostic tool may be different at baseline and after chemotherapy (Utzinger *et al.* 2000; Utzinger *et al.* 2002). It follows that at baseline, the 'true' prevalence might be underestimated while at treatment evaluation, the rate of false-negative results is higher, feigning exaggerated cure rates. This challenge has been encountered repeatedly in China where chemotherapy-based control programmes for *S. japonicum* have been maintained over several years and resulted in a preponderance of light infections that are difficult to diagnose by the Kato-Katz method alone (Yu *et al.* 1998; Wang *et al.* 2006a).

4.5.2 Commonly used diagnostic techniques for parasitological surveys

Most surveys on human intestinal parasites rely on the collection of a single stool sample and its analysis by one of a small number of diagnostic tests which are considered standard techniques. The most prominent tool for the detection of helminth eggs in stool samples is the Kato-Katz thick smear test (Katz et al. 1972), probably due to its simplicity, low material and infrastructure requirements, and cheap price. Intestinal protozoa are often diagnosed using concentration methods, e.g. ether-concentration after SAF-conservation (Marti and Escher 1990).

S. stercoralis, however, is not reliably diagnosed by the Kato-Katz thick smear, ether-concentration or direct smear techniques (Sato et al. 1995). The Koga agar plate (Koga et al. 1991) and Baermann technique (García 2007) are recommended instead (Vadlamudi et al. 2006) but marked fluctuations in the number of excreted larvae necessitate stool collection and analysis over several days (Nielsen and Mojon 1987).

4.5.3 Need for new diagnostic techniques

The ignorance about the true extent of multiparasitism can not last be attributed to the unavailability of a single diagnostic method which reliably detects all parasite species (Keiser *et al.* 2002). Currently, the only strategy to overcome this challenge is to focus on 1-2 biofluids, employ a range of diagnostic techniques, and screen multiple samples collected over consecutive days (van Gool *et al.* 2003). To overcome this time- and resource-straining

approach, sensitive, broad-spectrum diagnostic tools need to be developed. Little emphasis has been placed on this issue in recent years but the development of the FLOTAC method (Cringoli 2004, 2006) which detects intestinal helminths (Utzinger *et al.* 2008) and protozoa with high sensitivity holds promise to facilitate the evaluation of intestinal multiparasitism.

4.6 Global burden estimates, control programmes and significance of spatial statistics for risk prediction

4.6.1 Burden due to schistosomiasis and soil-transmitted helminthiasis

Untreated cases of schistosomiasis result in substantial morbidity and even mortality, but the extent is disputed. The estimated global burden of schistosomiasis is 1.7-4.5 million disability adjusted life years (DALYs) lost annually (WHO 2002; Utzinger and Keiser 2004; WHO 2004). However, a meta-analysis suggested a several-fold higher burden (King *et al.* 2005). One study attributed 280,000 annual deaths to *S. haematobium* and *S. mansoni* infections in sub-Saharan Africa alone (van der Werf *et al.* 2003). The morbidity and mortality associated with untreated schistosomiasis japonica is especially high, probably due to the higher number of eggs produced by *S. japonicum* (Gillespie and Pearson 2001; Ross et al. 2001). A recent study on the disability weight of chronic schistosomiasis japonica also suggested serious underestimation of the real burden in endemic populations (Jia *et al.* 2007).

Soil-transmitted helminthiasis remains among the most prevalent and debilitating parasitic diseases in the world (Horton 2003; Bethony *et al.* 2006; Hotez *et al.* 2006). The direct mortality was long assumed to be low, the World Health Report published in 2004 put the figure at 12,000 annual deaths (WHO 2004). However, other mortality estimates indicate 135,000 deaths per year (WHO 2002). Recent estimates suggest that the global burden caused by soil-transmitted helminthiasis might by as high as 39 million DALYs, thus approaching the burden of malaria (Hotez *et al.* 2007).

4.6.2 Burden due to neglected tropical diseases and recent initiatives for their control

The above said, it becomes clear that the true burden due to the so-called neglected tropical diseases, which include parasitic, bacterial, and viral diseases endemic in the tropics, is not known (Molyneux 2004; Engels and Savioli 2006). The burden inflicted on mankind by some neglected tropical diseases is certainly underestimated (de Vlas and Gryseels 1992; WHO 2002; King *et al.* 2005; Jia *et al.* 2007) while no burden estimates whatsoever have been calculated for other conditions included in this group (Engels and Savioli 2006; Hotez et al.

2006). Together, the burden due to neglected tropical diseases might surpass that of malaria or tuberculosis (Chan 1997; Hotez *et al.* 2007). Infectious diseases disproportionately affect developing countries (Ezzati *et al.* 2002; Lopez and Mathers 2006) and the burden due to neglected tropical diseases is borne almost exclusively by the socio-economically disadvantaged population segments of low- and middle-income nations (Gwatkin *et al.* 1999).

There is increasing appreciation of this high disease burden (Savioli *et al.* 2004a) and it has been noted that today, the control of some diseases like schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis has become simple (Bundy and de Silva 1998) and highly cost-effective (Molyneux 2004; Molyneux *et al.* 2005), is crucial for afflicted countries to free themselves from the poverty trap (Molyneux et al. 2005), and can benefit the control of other diseases, e.g. malaria (Molyneux and Nantulya 2004). In addition, chemotherapy rapidly reduces infection-related morbidity (Savioli *et al.* 2004b; Savioli *et al.* 2005). In response, a set of effective, mainly chemotherapy-based interventions for the rapid reduction of morbidity and community prevalences has been developed (WHO 2002; Albonico *et al.* 2006; WHO 2006; Hotez *et al.* 2007). Various large-scale mass drug administration (MDA) programmes for the control of neglected tropical diseases have already been launched (Southgate *et al.* 2005; Brady *et al.* 2006; Fenwick 2006; Fenwick *et al.* 2006; Kabatereine *et al.* 2006a). The evaluation of their true effect is challenging (Brooker *et al.* 2004) but these programmes are usually assumed to be highly effective and their success is probably not always adequately recognized (Molyneux 2004). However, the effects of chemotherapy-only programmes without ancillary activities to improve water supply and sanitation, supporting health education and general improvements of living conditions are probably not sustainable (Utzinger *et al.* 2003; Singer and de Castro 2007).

Integration of control programmes and linking them with efforts to curb other diseases like tuberculosis, malaria and HIV/AIDS has been advocated repeatedly (Molyneux and Nantulya 2004; Molyneux *et al.* 2005; Hotez *et al.* 2006; Lammie *et al.* 2006; Savioli *et al.* 2006; Utzinger and de Savigny 2006) and estimates suggest substantial cost savings (Brady et al. 2006). However, practical experience is still limited and theoretical as well as practical challenges remain (Kabatereine *et al.* 2006b; Richards *et al.* 2006; Kolaczinski *et al.* 2007).

Spatial targeting of control efforts is one of the main issues in large-scale control programmes. The spatial epidemiology of many neglected tropical diseases is known at country-level while information at district level, i.e. the operational unit of most health interventions, is often unavailable. The developments in the field of GIS technology, the availability of high-resolution RS data and the advances made in spatial risk prediction hold

promise to supplement more traditional disease mapping through surveys and to guide control programmes (Brooker and Michael 2000; Brooker 2002; 2007; Spear *et al.* 2002; Raso *et al.* 2005; 2006; Yang *et al.* 2005b; Beck-Wörner *et al.* 2007; Brooker and Utzinger 2007). GIS and RS have already been successfully applied to target MDA (Brooker *et al.* 2002; Kabatereine *et al.* 2004) and in integrated control programmes (Zhou *et al.* 2001; Yang *et al.* 2005b)

4.7 Treatment options, current strategies and development of new drugs

A limited choice of safe and efficacious drugs is currently available for the treatment of schistosomiasis, helminthiasis and most intestinal protozoa infections. However, none of the compounds covers the whole parasite spectrum and they were mostly developed decades ago (WHO 2002; Utzinger and Keiser 2004; Farthing 2006). The main anthelminthic drugs are praziquantel for the treatment of trematodes and cestodes, albendazole, mebendazole (both benzimidazol carbamates), levamisole and pyrantel pamoate for intestinal nematodes and ivermectin for *S. stercoralis* and lymphatic filariasis. Diethylcarbamazine is another standard drug to treat lymphatic filariasis. Single-dose treatment is very common (Anonymous 2004; Keiser and Utzinger 2004; Utzinger and Keiser 2004). Commonly used drugs against intestinal protozoa include metronidazole, nitazoxanide and co-trimoxazole, among others. Standard treatment courses usually involve repeated doses (Anonymous 2004; Farthing 2006).

The absence of individual diagnosis during MDA campaigns, the high prevalence of multiparasitism (see above) and the limited activity of many compounds against non-target parasites at commonly-used treatment schedules makes it likely that in the course of chemotherapy directed at a specific condition, further parasites are exposed to the drug, some of them at sub-curative doses. This gives rise to concerns about the development of resistance among human helminths, a serious problem in veterinary medicine. Thus far, however, no systematic reduction of the treatment efficacy of any anthelminthic agent has been observed in humans (Geerts and Gryseels 2000).

The current moves to integrate various chemotherapy programmes (see above) result in increasing co-administration of different anthelminthic compounds. Drugs against soil-transmitted helminths are now commonly distributed in the frame of lymphatic filariasis and schistosomiasis control, resulting in the co-administration of ivermectin or praziquantel and albendazole/mebendazole, respectively. Other drug combinations are also common (Brady et al. 2006). The safety and efficacy of co-administration of various drugs is of considerable

concern (Urbani and Albonico 2003) and has been assessed repeatedly. Empirical data from the field suggests no negative interactions between drugs (WHO 2006; Olsen 2007) and commonly low levels of adverse side effects even in areas of high multiparasitism levels and where non-target parasites are likely to be present (Utzinger and Keiser 2004; Olsen 2007). However, exceptions exist (García et al. 2003; Utzinger and Keiser 2004)

The small number of available anthelminthic compounds and the lack of innovation in drug development spurred concerns about the future of anthelminthic treatment once resistance against one of the major products develops among human parasites (Geerts and Gryseels 2000). The advent of tribendimidine, a new safe and efficacious broad-spectrum anthelminthic developed since the 1980s by Chinese scientists at the National Institute of Parasitic Diseases in Shanghai spurred considerable interest since this drug belongs to a new substance class and probably has a different mode of action. The Chinese drug authorities approved tribendimidine for medical use in humans in early 2004 (Xiao *et al.* 2005a). A standard single dose of tribendimidine efficiently cures *A. lumbricoides* and hookworm as well as *Enterobius vermicularis* infections. Triple doses are recommended for *T. trichiura* (Xiao et al. 2005a). Further experiments indicate activity of tribendimidine against cestodes (Xiao et al. 2005a), certain trematodes (Keiser *et al.* 2007) and *Strongyloides ratti* (Keiser *et al.* 2008) but the efficacy and treatment schemes for human application remain to be investigated for many parasites.

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5. Goals

The overarching goals of this Ph.D. thesis are (i) to update the global estimates pertaining to human schistosomiasis and to explore the effects of water resources development and management on its local endemicity; (ii) to determine the regional prevalence and epidemiology of different parasitic infections in Eryuan county, Yunnan province, China; (iii) to identify demographic, environmental and socio-economic risk factors governing the spatial distribution of *S. japonicum* seropositivity, and to generate smooth risk maps; (iv) to study intestinal multiparasitism and the local endemicity of *S. stercoralis* in two villages of Menghai county, Yunnan province, including assessment of the performance of diagnostic tools; and (v) to test of the safety and efficacy of tribendimidine against *S. stercoralis* and *Taenia* spp.

5.1 Specific objectives

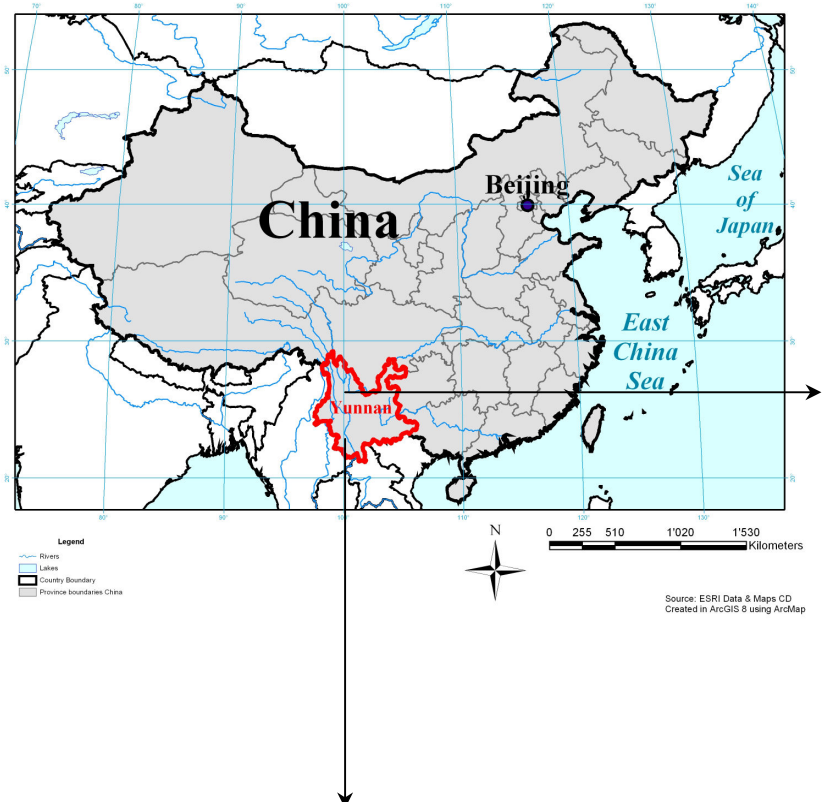
- To update country-specific estimates of the at-risk population and the number of people infected with schistosomes.
- To generate estimates of (i) the number of people at risk of schistosomiasis due to close proximity of irrigation schemes and large dam reservoirs; and (ii) pooled random risk ratios of schistosomiasis associated with their construction.
- To assess the prevalence and intensity of infection of *S. japonicum*, soil-transmitted helminths and food-borne helminths using standardized parasitological and serological diagnostic tests across Eryuan county, and to investigate behavioural, demographic, environmental and socio-economic risk factors for infection, and to set up a comprehensive GIS.
- To develop a spatially-explicit statistical model integrating epidemiological and remotely-sensed environmental data for risk profiling of *S. japonicum* seroconversion in Eryuan county and to explore the potential of serological data for the appreciation of the infection pressure.
- To assess the extent of intestinal multiparasitism and the local endemicity of *S. stercoralis* in Menghai county, and to study the influence of sampling effort and to investigate the performance of different diagnostic techniques.
- To assess the safety and efficacy of a single oral dose of tribendimidine for the treatment of (poly-)helminth infections, with an emphasis on *S. stercoralis* and *Taenia* spp., and to compare the results to standard treatment using albendazole.

6. Study sites

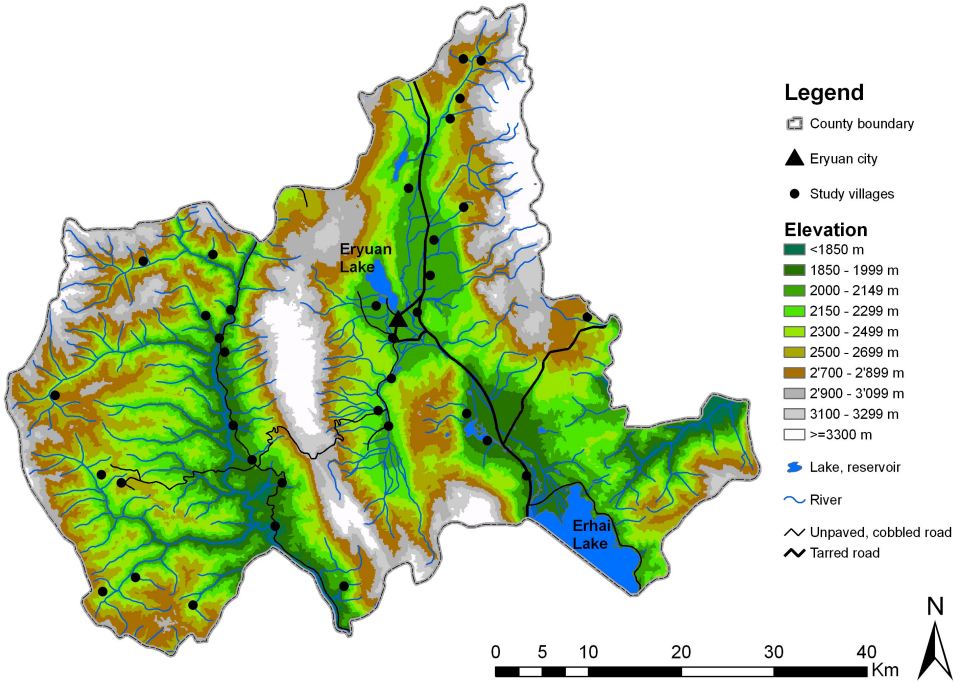
The fieldwork performed in the frame of this Ph.D. thesis was carried out in two settings in Yunnan province in southwest China.

A cross-sectional survey carried out in November and December 2005 covered 35 villages across Eryuan county in Dali prefecture, northwest Yunnan province (99.54° - 100.34° E longitude and 25.80° - 26.43° N latitude, ~3000 km^2, population 273,000 in 2005). Two distinct landscape types characterize this county. Mountain-framed plains with lakes at an elevation of 1950-2150 m occupy the eastern part of the county while forest-covered mountain ranges of over 3000 m elevation tower above deep valleys (lowest point: 1700 m) in the western section. The climate is characterized by monsoon rains in the summer half-year and dry chilly winters. The local majority ethnic group are the Bai people; the immigrated Han mainly inhabit the plain areas. Agriculture provides the economic basis of the county with cash-crop farming in the plains (rice, tobacco, garlic) and subsistence farming complemented by forestry and *Eucalyptus* oil production in the mountainous areas. Livestock is gaining prominence; the main species are cows (milk production; in plains), cattle (meat; mountains), buffaloes (work), pigs, horses or mules (work) and poultry. Dogs are almost ubiquitous.

Two community-based studies were conducted in the villages of Nongyang (100.35° E longitude and 21.81°N latitude, elevation 1350 m) and Nanweng (100.40° E longitude and 21.77° N latitude, elevation 1650 m) in Menghai county in the southwestern Xishuangbanna prefecture of Yunnan province. These surveys were carried out in May 2006 and May-June 2007, respectively. The subtropical climate is governed by abundant rainfall during the summer monsoon time and dry warm winter months. Both villages are situated in hilly area and inhabited by ethnic Bulang people. Tea plantations provide the monetary income for the village population, complemented by subsistence farming including livestock breeding (dogs, pigs, buffaloes).



Nongyang village
Nanweng village
Menghai County
Xishuangbanna Dai Autonomous Prefecture



Eryuan County
Dali Bai Autonomous Prefecture

7. Schistosomiasis and water resources development: systematic review, meta-analysis and estimates of people at risk

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7.1 Abstract

An estimated 779 million people are at risk of schistosomiasis, of whom 106 million (13·6%) live in irrigation schemes or in close proximity to large dam reservoirs. We identified 58 studies that examined the relation between water resources development projects and schistosomiasis, primarily in African settings. We present a systematic literature review and meta-analysis with the following objectives: (1) to update at-risk populations of schistosomiasis and number of people infected in endemic countries, and (2) to quantify the risk of water resources development and management on schistosomiasis. Using 35 datasets from 24 African studies, our meta-analysis showed pooled random risk ratios of 2·4 and 2·6 for urinary and intestinal schistosomiasis, respectively, among people living adjacent to dam reservoirs. The risk ratio estimate for studies evaluating the effect of irrigation on urinary schistosomiasis was in the range 0·02-7·3 (summary estimate 1·1) and that on intestinal schistosomiasis in the range 0·49-23·0 (summary estimate 4·7). Geographic stratification showed important spatial differences, idiosyncratic to the type of water resources development. We conclude that the development and management of water resources is an important risk factor for schistosomiasis, and hence strategies to mitigate negative effects should become integral parts in the planning, implementation, and operation of future water projects.

7.2 Introduction

This review is the fourth of a series of systematic literature reviews pertaining to water resources development and management and its effects on water-associated diseases. The previous reviews covered three water-related vector-borne diseases, namely malaria,[1] lymphatic filariasis[2] and Japanese encephalitis.[3] The current focus is on the most important water-based disease from a global public health perspective - i.e., schistosomiasis.

Known since ancient times,[4] schistosomiasis ranks second only to malaria among the parasitic diseases with regard to the number of people infected and those at risk. According to previous estimates, the disease causes the annual loss of between 1·7 and 4·5 million disability adjusted life years (DALYs).[5-7] A recent meta-analysis challenges these burden estimates; they could be several-fold higher.[8] Most of the present schistosomiasis burden is concentrated in sub-Saharan Africa[9] with the highest prevalence and infection intensities usually found among school-age children, adolescents and young adults.[10,11] Schistosomiasis negatively impacts on school performance and the debilitation caused by untreated infections undermines social and economic development in heavily affected areas.[12-15]

One way to meet the increasing food and energy demands of the growing world population is through the construction of dams and irrigation schemes. Irrigated agriculture usually results in increased crop outputs, and hydropower reduces dependency on domestic or imported fossil fuels and generates export earnings. In addition, reservoirs are one way to address water scarcity through increased storage capacity.[16] Water resources development takes place in most parts of the world, at different scales and at a rapid pace. Over 33 000 dams are listed in the latest edition of the World Register of Dams; 3000 of them were built in the 1990s.[17] The total area under irrigation was 277 million ha in 2002; an increase of almost 10% over the past 10 years.[18]

However, the development and management of water resources in tropical and sub-tropical climate zones has often resulted in transmission intensification or the introduction of diseases into previously non-endemic areas.[19-22] Schistosomiasis is considered a sensitive indicator disease for monitoring ecological transformations as it is widely distributed and infection rates can change promptly.[23]

The objectives of the present review are (1) to update estimates of at-risk populations and number of people infected with schistosomes in endemic countries, and (2) to estimate the number of people at risk of the disease due to close proximity of irrigated areas and large dam reservoirs. In addition, we identify generic features of the changing epidemiology of

schistosomiasis following implementation and operation of water resources development projects and provide pooled random risk ratios of schistosomiasis associated with dam and irrigation scheme construction.

7.3 Methods

7.3.1 Search strategy and selection criteria

A systematic literature review was done with the aim to identify all relevant studies that examined the effects of water resources development and management on schistosomiasis.

We did computer-aided searches of the following electronic databases: PubMed, BIOSIS preview, Web of Science, Science Direct, Literatura Latino Americana e do Caribe em Ciências da Saùde (LILACS), ArticleSciences, and African Journals OnLine (AJOL). Next, we searched the electronic archives of international organizations - i.e., WHO, the Food and Agricultural Organization (FAO), and the World Bank. Books, dissertations and unpublished documents ("grey literature") were also considered. The following keywords and combinations thereof were used: "schistosomiasis" in combination with "dam(s)", "barrage", "impoundment", "reservoir(s)", "pool(s)", "flood control", "irrigation", "paddy rice", "swamp rice", "water management", "environmental management" and "ecological transformation". Neither temporal limits nor language restrictions were set for database searches. The bibliographies of all recovered documents were hand-searched for additional references.

The decision tree for the inclusion or exclusion of articles is shown in Figure 7.1. Only publications reporting pre-development and post-development schistosomiasis prevalence data from one area, or cross-sectional data obtained from otherwise comparable settings with specified differences in water resources development and management, were included. Articles reporting only pre-development prevalences were included if case matching follow-up publications could be identified.

7.3.2 People at risk of schistosomiasis and number of people infected

The number of people at risk of schistosomiasis and those infected in schistosome-endemic countries at mid-year 2003 were obtained as follows. First, whenever possible, the country estimates of the at-risk population[24-29] and numbers of people infected[24-45] were obtained from the latest available surveys. Second, for countries where no recent data were available, the proportions of people "at risk" and those actually "infected" as of 1995 were calculated from

numbers presented by Chitsulo and colleagues[9] and applied to the United Nations (UN) total population estimates by mid-year 2003.[46]

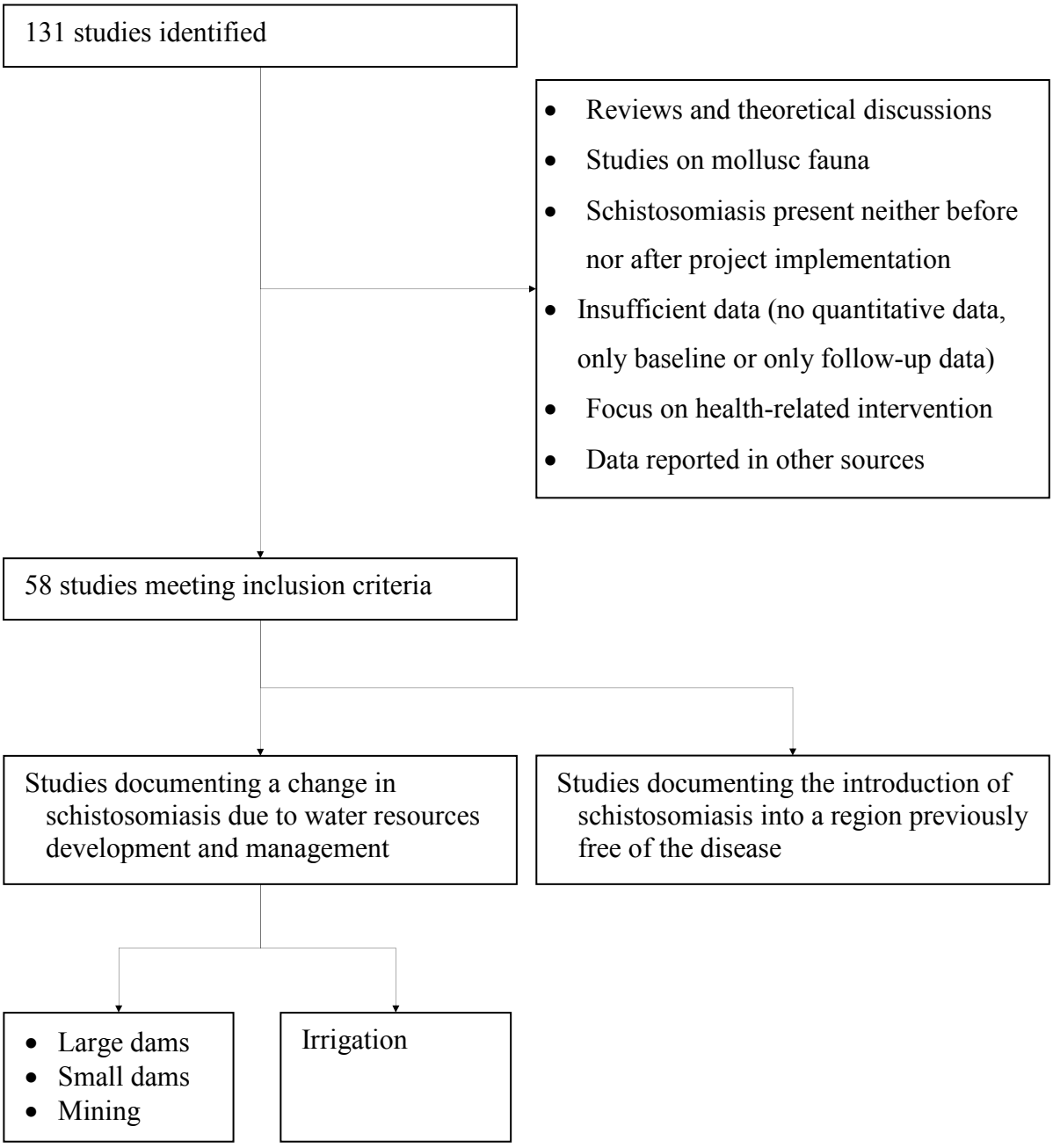

Figure 7.1 Decision tree showing inclusion and exclusion of studies identified on the interface of schistosomiasis and water resources development and management

7.3.3 Proximity to irrigated agriculture

Information on total agricultural area (sum of arable land, permanent crops and permanent pasture[18]) and area under surface irrigation[47] was obtained for all schistosome-endemic

countries. Data on rural population numbers were obtained from the UN.[46] The population density in arable areas was calculated by dividing the total rural population of the country by its total area classified as “arable” or “planted with permanent crops”. A similar population density was assumed for irrigated areas. Due to the lack of data on the distribution of irrigated agriculture with regard to schistosome-endemic areas, the number of people at risk of schistosomiasis in irrigated areas (irrigated population at risk) was estimated by multiplying the surface-irrigated area by the mean population density in arable areas of the respective country and the national population fraction at risk of schistosomiasis (Figure 7.2).

Figure 7.2 Partly lined irrigation canal in Yunnan province, China, where *Schistosoma japonicum* is endemic (Picture: Swiss Tropical Institute)

7.3.4 Proximity to dam reservoirs

All data on reservoirs of large dams (i.e., height ≥15 m and/or storing volume >3 million m^3) were derived from the World Register of Dams.[17] The assumptions and calculations underlying the estimate of the at-risk population due to proximity to large dam reservoirs are detailed in our previous work focusing on malaria.[1] The following adjustments were made. First, the distance from the lakeshore was extended from 2 km to 5 km. Justification for this cut-off value arises from studies on *Schistosoma mansoni* around Lake Victoria.[24,48] Thus, the formula for calculating the approximate area at risk (a hollow rectangle, having rounded corners, which surrounds the water body) became: 2 x (length x 5) + 2 x (width x 5) + $5^2\Pi$. Second, we stratified all reservoirs 1 km^2 or larger on the schistosome-endemic continents of Africa and South America (all reservoirs) and Asia (reservoirs ≥10 km^2) for which information on both reservoir surface and reservoir length was available according to surface area and calculated their median length, width, and at-risk area. A square-shape was assumed for reservoirs smaller than 1 km^2.

Subsequently, we determined the number of dams for each schistosome-endemic country, stratified the lakes by surface area and multiplied their number with the respective area at risk. The mean area at risk per lake was used for reservoirs for which information on surface area was unavailable. The country-specific at-risk population due to proximity to large dam reservoirs was obtained as detailed for the irrigated areas.

7.3.5 Statistical analysis

We stratified the studies according to schistosome species and type of water resources development - i.e., studies in irrigated areas were classified as “irrigation” even if the irrigation water was supplied by an artificial lake. Data for population sub-groups (prevalence, sample size) were extracted and analysed with version 2.4.5 of StatsDirect software (StatsDirect Ltd, Chesire, UK). Risk ratios and corresponding 95% CIs were calculated. Heterogeneity between studies was determined with Cochrane’s Q statistics. A random effect model was used for the summary risk ratio, because the test of heterogeneity was highly significant ($p<0{\cdot}001$). Whenever consecutive results from a single study were available, only the most recent data point was considered. Furthermore, only the data from the project area with the most extensive water development activity was used.

7.4 Results

The creation of dam reservoirs and the implementation of irrigation systems often lead to an expansion of the habitats of intermediate host snails, and hence new potential transmission ites for schistosomiasis. Improvements in water supply and sanitation, on the other hand, can break the transmission cycle through reduced human-water contact and diminished environmental contamination with excreta. In addition, there are ancillary benefits of such improved water-related infrastructure.[49] Water resources development can result in better socioeconomic conditions with additional resources available for health-related interventions - e.g., procurement of efficacious antischistosomal drugs such as praziquantel.

7.4.1 Current global status of schistosomiasis

According to WHO, schistosomiasis is endemic in 76 countries and territories.[50] The disease also occurs in Djibouti, where it probably was introduced by refugees[51,52] and its presence in Nepal is suspected.[53] Active transmission is reported from 67 countries and territories, down from 71 in the mid-1990s.

For mid-year 2003 we estimate that 779 million people were at risk of schistosomiasis, and 207 million people were infected (Appendix 7.1 and Appendix 7.2). Large-scale morbidity control programmes, socioeconomic development, and environmental changes, including the sometimes deliberate introduction of competitor snail species, have resulted in transmission interruption or disease elimination in nine countries (Iran, Japan, Lebanon, Malaysia, Martinique, Montserrat, Thailand, Tunisia, and Turkey), and considerable reductions of people infected and disease-attributable morbidity in Brazil, China, Egypt, Morocco, the Philippines, Venezuela,[50] countries of the Caribbean,[54] as well as Cambodia and Laos.[27] The reported or estimated number of infections is below 1000 in Antigua and Barbuda, India, Indonesia, Jordan, Oman and Saint Lucia. In some countries - e.g., Jordan - most detected cases are imported rather than autochthonous.[39]

Of all countries with ongoing transmission, 46 are in Africa. They are home to about 97% of all infections and 85% of the global population at risk. At present, 29 African countries, Brazil and Yemen harbour more than one million cases each.

7.4.2 Irrigation, dams, and schistosomiasis stratified by WHO subregions

The estimates of the number of people at risk of schistosomiasis due to irrigation and large dam reservoirs, stratified by WHO subregions, are summarized in Table 7.1. Overall, 58

articles reporting data from 39 settings met our inclusion criteria. Table 7.2 summarises the effects of irrigation on schistosomiasis.[55-84] Table 7.3 shows the relation between large and small reservoirs, as well as fishponds, and the disease.[62,85-103] Introduction of schistosomiasis into previously non-endemic areas following water resources development and mining activities is summarised in Table 7.4.[55,63,65,93,94,99-113]

Table 7.1 Summary of schistosomiasis burden (expressed in DALYs lost), numbers of people infected, and at-risk population due to proximity to water resources development and management, stratified by WHO subregion

WHO subregion	Schistosomiasis burden expressed in DALYs lost in 2002 (%)[a]	No. of people infected with *Schistosoma* spp. by mid-2003 (%)[b]	No. of people at risk of schistosomiasis by mid-2003 (%)b	No. of people at risk of schistosomiasis living in irrigated areas (%)[c]	No. of people at risk of schistosomiasis living in proximity to large dam reservoirs (%)c	Fraction of population at risk living in proximity to:	
						Irrigated areas	Dams
1	621 (36·6)	98 118 (47·3)	275 404 (35·3)	6082 (9·6)	6162 (14·5)	2·2%	2·2%
2	713 (42·0)	89 066 (43·0)	283 344 (36·4)	3923 (6·2)	22 543 (53·2)	1·4%	8·0%
4	74 (4·4)	1809 (0·9)	36 033 (4·6)	537 (0·8)	1220 (2·9)	1·5%	3·4%
6	29 (1·7)	306 (0·1)	12 629 (1·6)	941 (1·5)	209 (0·5)	7·5%	1·7%
7	197 (11·6)	16 920 (8·2)	125 344 (16·1)	42 627 (67·4)	2129 (5·0)	34·0%	1·7%
9	0 (<0·1)	0 (<0·1)	72 (<0·1)	4 (<0·1)	7 (<0·1)	5·6%	9·7%
11	3 (0·2)	0 (<0·1)	108 (<0·1)	8 (<0·1)	2 (<0·1)	7·4%	1·9%
12	4 (0·2)	0 (<0·1)	13 (<0·1)	3 (<0·1)	3 (<0·1)	23·1%	25·5%
13	0 (<0·1)	0 (<0·1)	Eliminated	-	-	-	-
14	55 (3·2)	1065 (0·5)	46 371 (5·9)	9125 (14·4)	10 081 (23·8)	19·7%	21·7%
Total	**1696 (100)**	**207 284 (100)**	**779 318 (100)**	**63 250 (100)**	**42 356 (100)**	**8·1%**	**5·4%**

[a] $x10^3$, source: WHO[5]
[b] $x10^3$, based on Chitsulo and colleagues.[9], updated from various sources and recalculated with figures from UN[46]
[c] $x10^3$

Table 7.2 Identified studies on schistosomiasis in irrigated areas, stratified by WHO subregion

Country, project, design, and period	Ref.	Sample size	Age-group	Spe-cies	Prevalence		Risk ratio (95% CI)	Comment
					No irrigation	Irrigation		
WHO subregion 1								
Burkina Faso, Sourou, 1954 *vs.* 1998/99	55	Not given	SC	*S. h.*	19%	8·5-70·3%	NA	Léry dam closed: 1976 Main canal opened: 1985
Burkina Faso, Kou valley, 1957 *vs.* 1987	55	Not given	SC SC	*S. h.* *S. m.*	14% 1·3% (1987)	80% 45% (1998)	5.7 NA	Start of irrigation: 1968
Burkina Faso, Kaya, cross-sectional, end 1970s	56	Swamp: 120 Natural lake: 800 Irrigation: 1500	TP TP	*S. h.* *S. m.*	Swamp: F = 25%, M = 40·5% Lake: F = 9%, M = 18% Swamp: F = 14·5%, M = 49% Lake: F=23%, M=31·5%	 F = 8%, M = 12% F = 19%, M = 21%	 - -	Start of irrigation: 1967/68
Cameroon, SEMRY II, cross-sectional, 1980	57	Not irrigated: 174 Irrigated: 816	TP	*S. h.*	20·1%	48·5%	2·4 (1·8-3·3)*	Start of irrigation: 1971 Study period: 1980
1979-85	58	March 1979: 1780 Nov. 1979: 1558 May 1981: 1458 Nov. 1981: 1139 April 1985: 1661	TP	*S. h.*	March 1979: 5·2%	 Nov. 1979: 6·5% May 1981: 6·2% Nov. 1981: 5·7% April 1985: 5%	 1·2 (0·9-1·6) 1·2 (0·9-1·6) 1·1 (0·8-1·5) 1·0 (0·7-1·3)*	Impoundment of lake: 1979 Improved sanitation, decreased rainfall
Liberia, Bong county, cross-sectional, 1980	59	No swamp: 174 Swamp: 423 No swamp: 168 Swamp: 384	TP TP	*S. h.* *S. m.*	11% 9%	42% 87%	3·6 (2·5-6·0)* 9·7 (6·0-15·8)*	Swamp rice started: 1974 Villages 50 km apart
Madagascar, Ankilivalo, 1971 *vs.* 1986 and cross-sectional, 1986	60	1971: - 1986: 279 Distant village: 84 Close village: 413	SC SC	*S. m.* *S. m.*	13% 7%	74% 69·2%	5·7 9·7 (4·5-21·0)*	Morondava dam closed: 1979 Distance between villages: 8 km
1986 *vs.* 1994	61	1986: 413 1994: 369	SC	*S. m.*	34·1%	69·2%	2·0 (1·7-2·4)*	Dam destroyed: 1990 Prevalence among 79 SC in distant village: 7·6%

Country, project, design, and period	Ref.	Sample size	Age-group	Spe-cies	Prevalence		Risk ratio (95% CI)	Comment
					No irrigation	Irrigation		
Mali, general, cross-sectional, 1980 et sqq.	62	Savannah: 10 744 Irrigated: 8955 Savannah: 7776 Irrigated: 6146	TP TP	*S. h.* *S. m.*	13·4% 1·6%	64·4% 53·9%	4·8 (4·6-5·1)* 33·8 (28·3-40·3)*	Data from national survey
Senegal, Senegal River, baseline 1977/78	63	Delta: 214 "walo": 803 "diéré" 375	SC	*S. h.*	Delta: 0% "walo": 0·7% "diéré": 10·4%			Diama dam closed: 1985 Manantali dam closed: 1993
Baseline 1985	64	Delta: 1441 "walo": 1653 "diéré": 327	SC	*S. h.*	Delta: 0·9% "walo": 3·6% "diéré": 27·2%			Prevalence in 246 people from delta villages with irrigation: 29·7%
Cross-sectional, 1994/95	65	Delta: 2920 "walo": 2,585 "diéré": 1,011	TP	*S. h.*		Delta: 1·9-41·1% (4/23-18/21) "walo": 11·5% (20/59) "diéré": 51·6% (2/28)	- - -	Percentages refer to endemic villages. Values in brackets refer to the number of endemic/number of total villages Prevalence in 1139 people from Lac de Guiers: 29·7% (1/18)
Senegal, Upper valley, cross-sectional, 1997-99	66	1997: 835 with irrig. 610 without irrig. 1999: 373 with irrig. 382 without irrig.	SC	*S. h.*	1997: 27·0% 1999: 20·2%	1997: 48·0% 1999: 78·3%	1·8 (1·5-2·1) 3·9 (3·2-4·8)*	Increase in irrigated area between 1997 and 1999 in some locations
Sierra Leone (southeast), cross-sectional, end 1970s	67	No swamp: 221 Swamp: 6668 No swamp: 146 Swamp: 4840	TP TP	*S. h.* *S. m.*	11·7% 4·7%	8·1% 2·4%	0·7 (0·5-1·0)* 0·5 (0·2-1·1)*	Swamp rice. 6 villages without and 68 villages with swamps Gbakima[68]: 1106 people in areas with swamps, prevalence S. h.: 0·6%, S. m.: 0·3%
WHO subregion 2								
Côte d'Ivoire, savannah and forest, cross-sectional, 1997-99	69	966-1420	SC SC	*S. h.* *S. m.*	Savannah: 0·7% Forest: 1·7% Savannah: 2·1% Forest: 17·5%	Savannah: 2·3/4·8% Forest: 4·4/0·9% Savannah: 11·9/16·1% Forest: 46·6/61·3%	3·5 (1·5-7·8)/ 7·4 (3·4-16·0)* 2·5 (1·5-4·3)/ 0·5 (0·3-1·1)* 5·7 (3·6-9·1)/ 7·8 (5·0-12·2)* 2·7 (2·3-3·1)/ 3·5 (3·0-4·0)*	Inland valleys with and without swamp rice, those with swamp rice with partial (first number)/full (second number) water control
Tanzania, Mbarali, 1962 *vs.* 1965	70	1962: 63; 1965: 263 1962: 62; 1965: 263	TP TP	*S. h.* *S. m.*	9·5% 14·5%	8·7% 28·9%	0·9 (0·4-2·1)* 2·0 (1·1-3·8)*	Start of settlement: 1961

Country, project, design, and period	Ref.	Sample size	Age-group	Spe-cies	Prevalence		Risk ratio (95% CI)	Comment
					No irrigation	Irrigation		
WHO subregion 7								
Egypt, Difra, 1935 *vs.* 1979	71	1935 and 1979: 315	TP	*S. h.*	74·3%	2·2%	0·03 (0·01-0·06)*	Aswan low dam closed: 1933
			TP	*S. m.*	3·2%	73%	23·0 (12·5-42·5)*	Aswan high dam closed: 1970
Egypt, Nile delta, 1935 *vs.* 1983	72	1935: 14 815	TP	*S. h.*	56%	5%	0·09 (0·08-0·1)*	Aswan low dam closed: 1933
		1983: 15 166	TP	*S. m.*	33%	39%	1·18 (1·15-1·22)*	Aswan high dam closed: 1970
Egypt, Aswan and Quena provinces, 1934 *vs.* 1937	73	Not given	-	*S. h.*	2-11%	44-75%	NA	Shift from basin to perennial irrigation
Egypt, Assiut, Quena and Suhag provinces, cross-sectional, 1955	74	Basin irrigation: 47 748 Perennial irrigation: 62 006	-	*S. h.*	Basin irrigation: 16·2%	Perennial irrigation: 63·9%	3·9 (3·8-4·0)*	Basin versus perennial irrigation
Egypt, Bitter lakes area, 1985 *vs.* 1992	75	Not given	-	*S. h.*	7·8%	1·7%	0.2	Land reclamation
			-	*S. m.*	21·7%	42·1%	1.9	Only people settled ≥7 years
Sudan, Gezira, 1926-39 *vs.* 1942-45	76	1926-39: various nos.						Sennar dam closed: 1925
		S. h. 1942-45: 4773	-	*S. h.*	<1%	30%	30; baseline 1%	
Late 1940/50s		S. m. 1944: 3596	-	*S. m.*		1·3%	-	Taken as indication of baseline
1973	77	81 027	TP	*S. h.*		8·9%	8·9; baseline 1%	
			TP	*S. m.*		8·8%	4·4; baseline 2%	
1973/74	78	1655	TP	*S. h.*		0·2%	0·2; baseline 1%	Roseires dam closed: 1960
End 1970/80s			TP	*S. m.*		49·3%	24·7; baseline 2%	
	79	1608	SC	*S. m.*		82·1%	41·1; baseline 2%	
1981/82	80	229	TP	*S. h.*		17·9%	17·9; baseline 1%	
			TP	*S. m.*		67·7%	33·9; baseline 2%	
1983	81	1981: 3486	TP	*S. m.*		51%	25·5; baseline 2%	
		1982: 3521	TP	*S. m.*		61%	30·5; baseline 2%	
1986/87	82	4481	TP	*S. h.*		20·8%	20·8; baseline 1%	Only villages with haematuria in schools
			TP	*S. m.*		47·4%	23·7; baseline 2%	
	83	3648	TP	*S. h.*		10-15%	-	
			TP	*S. m.*		52%	26·0; baseline 2%	
WHO subregion 4								
Brazil, Bahia, cross-sectional 1986/94	84	No irrigation: 96 ≥500 ha irrigated: 40	TP	*S. m.*	Prevalence <5%: 40·6% Prevalence ≥20%: 8·8%	Prevalence <5·0%: 50·0%		Mean prevalence data of villages compared
			TP	*S. m.*		Prevalence ≥20·0%:5·0%	0·27	

S. h.: *Schistosoma haematobium*; S. m.: *Schistosoma mansoni*; TP: total population; SC: school-age children; F: female; M: male; irrig.: irrigation; NA: not applicable; *: included in meta-analysis

Table 7.3 Identified studies on schistosomiasis in proximity to dam reservoirs, stratified by WHO subregion

Country, project, study, and period	Ref.	Sample size	Age-group	Spe-cies	Prevalence		Risk ratio (95% CI)	Comment
					No dam	Dam		
WHO subregion 1								
Cameroon, Lagdo lake, cross-sectional, 1986	85	Distant: 188 Close: 1145 Distant: 185 Close: 964	TP TP	*S. h.* *S. m.*	13% 3%	26% 15%	2·0 (1·4-2·9)* 5·5 (2·3-13·3)*	Lagdo dam closed: 1982 Distant village: 20 km away from lake
Cameroon, Bafia, fish pond, cross-sectional	86	Distant: 89 Close: 245	TP	*S. m.*	7·8%	21·2%	2·7 (1·3-5·7)*	Fish pond in one quarter of city
Ghana, Lake Volta, 1959-61 *vs.* 1968 1967 1968 1974	87 88	1959-61: not given 1968: 442 1967: 1114 1968: 968 1974: 2824	SC SC TP	*S. h.* *S. h.* *S. h.*	1%	70·1% 1967: 29·8% 1968: 85·6% 1974: 73·4%	NA 17·1 14·7 (baseline 5%[87])	Akosombo dam closed: 1965 Village: Kete Krachi During impoundment, villages: Ampem, Amete, Kete Krachi 26 communities at Pawmpawm and Afram branch of the lake
Ghana, below Kpong dam, 1972 *vs.* 1980s	89	1972: not given 1980s: 59 (Bator), 50 (Mepe)	SC	*S. h.*	Bator: 27·1% Mepe: 36·4%	Bator: 74·6% Mepe: 88·0%	2.8 2·4	Kpong dam closed: 1981
Ghana, Barekese dam, 1966 *vs.* 1999	90	3 exposed and 1 control village, 18 households each (180-255 people)	TP	*S. h.*	1966, exposed: 17·5%	1999, exposed: 36·9%	2·1 (1·8-2·1)*	1966: pre-construction 1999: late operational phase Control: 0% in 1966/72 and 1·1% in 1999 (imported cases)
Ghana, upper east region, small dams, cross-sectional 1960/61	91	Without dams: 15 districts With dams: 23 districts	TP	*S. h.*	Median: 17% Mean: 19·8%	Median: 50·5% Mean: 45·3%	3·0	Prevalence data of districts compared Dams constructed 1951-1965
Mali, general, cross-sectional, 1980 et sqq.	62	Savannah: 10 744 Small dams: 3289 Sélingué: 3140 Savannah: 7776 Small dams: 2841 Sélingué: 2132	TP TP	*S. h.* *S. m.*	13·4% 1·6%	Small dams: 67·2% Sélingué: 31·8% Small dams: 12·0% Sélingué: 4·4%	5·0 (4·8-5·3)* 2·4 (2·2-2·6)* 7·5 (6·2-9·2)* 2·8 (2·1-3·6)*	Data from national survey
Mali, Sélingué dam area, 1980 *vs.* 1985	92	1980: not given 1985, S. h.: 1884 1985: S. m.: 1216	TP TP	*S. h.* *S. m.*	3·2% 0·9%	31·4% 5·8%	NA 6·4	Sélingué dam closed: 1980

Country, project, study, and period	Ref.	Sample size	Age-group	Spe-cies	Prevalence		Risk ratio (95% CI)	Comment
					No dam	Dam		
WHO subregion 2								
Côte d'Ivoire, Lake Taabo, 1976/77 *vs.* 1992	93,94	1976/77: 120 1992: 134	SC	*S. m.*	3%	2%	0·7 (0·2-2·9)*	Taabo dam closed: 1979 Baseline data: one village Follow-up data: five villages
Côte d'Ivoire, Lake Kossou, 1970 *vs.* 1992	93	1970: 1031 1992: 290	SC	*S. h.*	13·7%	53%	3·9 (3·2-4·7)*	Kossou dam closed: 1972 Baseline data: 4 villages Follow-up data: 5 villages
Democratic Republic of the Congo, Lwiro, 43 fish ponds, 1996	95	Distant: 397 Close: 390	TP	*S. m.*	4%	6·1%	1·5 (0·8-2·8)*	Ponds built: 1988 10% of inhabitants of distant villages also work in ponds
Ethiopia, Tigray, small dams, cross-sectional, 1997	96	Distant: 337 Close: 341	SC	*S. m.*	29·7%	48·4%	1·6 (1·3-2·0)*	Distant: 8-10 km, close: <3 km Incidence not significantly different
Nigeria, Ruwan Sanyi dam, 1971-79	97,98	1971: 199 boys 1976: 194 boys 1979: 217 boys	SC	*S. h.*	1971: 59·3% 1975: 33·8% 1976: 41·2%	55·8%	0·9 (0·8-1·1)*	Dam closed: 1977 1973: drought, made responsible for decline, afterwards normalization?
Zambia, Lake Kariba, Siavonga, 1968	99	1968: 726	TP	*S. h.*	low incidence before 1956[100]	28·8%	5·8; baseline 5%	Kariba dam closed: 1959 Intermediate host snail absent from construction site
Siavonga and Matinangala, 1991	101	516	TP	*S. h.*		16·7%	3·3; baseline 5%	
Siavonga, 1994	102	338	TP	*S. h.*		35·3%	7·1; baseline 5%	
Siavonga, 2002	103	527	SC	*S. h.*		19·4%	3·1; baseline 5%	

S. h.: *Schistosoma haematobium*; *S. m.*: *Schistosoma mansoni*; TP: total population; SC: school-age children; NA: not applicable; *: included in meta-analysis

Table 7.4 Identified studies reporting the introduction of schistosomiasis into an area following water resources development and management, stratified by WHO subregion

Country, project and period	Ref.	Sample size	Age-group	Spe-cies	Baseline confirming absence	Prevalence after water resources development	Comment
WHO subregion 1							
Burkina Faso, Sourou, irrigation scheme, 1999	55	Not given	SC	*S. m.*	First case diagnosed: 1987	8-69%	Léry dam closed: 1976 Main canal opened: 1985
Cameroon, Yaoundé, fishponds, 1960	104	61	-	*S. m.*	Not known before by inhabitants	64%	
Nigeria, Oyan river dam, 1988/89 1991/92	105 106	1988/89: 355 1991: 650 1992: 591	TP TP TP	*S. h.* *S. h.* *S. h.*	Not known before by inhabitants	1988/89: 80·6% 1991: 35·5% 1992: 61·6%	Oyan river dam closed: 1984 Treatment of 1988/89-study participants
Senegal, Senegal river basin, cross-sectional, 1994/95	65	Delta: 2920 "walo": 2585 "diéré": 1011	TP	*S. m.*	692 samples from delta, "walo" and "diéré" in 1977/78: S. m. 0%[63]	Delta: 4·4-43·6% (3/17-22/23) "walo": 0% "diéré": 0%	Diama dam closed 1985 and Manantali dam closed 1993. Percentages refer to endemic villages. Values in brackets represent the number of endemic villages/number of total villages. *S. m.* prevalence among 1139 people from Lac de Guiers villages: 71·8% (18/18)
Sierra Leone, Yengema town, pits from diamond mining, 1980s	107	451	TP	*S. m.*	First case diagnosed 1970	27·5%	Alluvial diamond mining and rice swamps, snails abundant in abandoned workings and in rice swamps
WHO subregion 2							
Côte d'Ivoire, Lake Taabo, 1992	93,94	258	SC	*S. h.*	120 samples in 1976/77: S. h.: 0%[94]	73%	Taabo dam closed: 1979 Baseline data from one village, follow-up data from five villages
Democratic Republic of the Congo, open cast tin mining, 1980s	108	6433	TP	*S. m.*	Known to be absent until about 1960	87·4%	Mining started: 1932 Data from heavily infected villages. Mean prevalence in area is 10-15%

Country, project and period	Ref.	Sample size	Age-group	Spe-cies	Baseline confirming absence	Prevalence after water resources development	Comment
Ethiopia, Awash valley, irrigation, 1973-76	109	Upper valley: 1516 Middle valley: 992	TP	*S. m.*	Believed to be absent throughout the region	Upper valley: 9·0% Middle valley: 2·0%	Start of large-scale irrigation: 1950s
Ethiopia, Awash valley, Wonji sugar estate, 1980	110	1980: 2251	TP	*S. m.*	First case diagnosed: 1964	20% (1968: 7·5%, 1972: 9%, 1975: 17%)	Irrigation started: 1954
1988	111	1988: 267	SC			1988: 81·9%	Children of one labour village
Kenya, Mwea irrigation scheme	112	1966: 1875 1971: 2978	-	*S. m.*	Not reported in area	1966: 12·5% 1971: 24·4% 1982: 25%	Irrigation started: 1952, first case diagnosed: 1959 Prevalence after temporary cessation of mollusciciding (1972): 40%
Zambia, Lake Kariba Siavonga, 1968	99	1968: 726	TP	*S. m.*	*S. m.* or intermediate host snail not mentioned[100]	1968: 5·9%	Kariba dam closed: 1959
Siavonga and Matinangala, 1991	101	1991: 474	TP	*S. m.*		1991: 54%	
Siavonga, 1994	102	1994: 323	TP	*S. m.*		1994: 60·1%	
Siavonga, 2002	103	2002: 391	SC	*S. m.*		2002: 33·5%	
WHO subregion 4							
Puerto Rico, Guayama/ Arroyo region, sugar cane irrigation	113	1906/1909: 623/2612 1930-50: 400-4000 1960: 1127 1965: 1693	TP	*S. m.*	0% (1906/09)	6-40%, generally about 25% (1930-50) 6% (1960) 1% (1965)	Irrigation started: 1914, Carite and Patillas reservoirs constructed: 1916 Control started in 1950s. Prevalences calculated for 6-year-old children

S. h.: *Schistosoma haematobium*; S. m.*: Schistosoma mansoni*; TP: total population; SC: school-age children

7.4.2.1 WHO subregions 1 and 2 (sub-Saharan Africa)

Approximately 49 000 km^2 are currently under surface irrigation in sub-Saharan Africa, which translates to only 3% of the total arable area. More than half of the area under surface irrigation is located in South Africa and Madagascar. The irrigated population at risk of schistosomiasis is estimated at 10 million or 1·8% of the local population at risk.

According to the World Register of Dams, 1506 large dams have been built in these two WHO subregions. We estimate that an area of 205 000 km^2 lies within 5 km of the lakeshores; approximately half is located in South Africa and Zimbabwe. We estimate that 28·7 million people at risk live within this area, representing 5·1% of the at-risk population. No data on large dams were available for Burundi, Central African Republic, Chad, Equatorial Guinea, Eritrea, The Gambia, Guinea-Bissau, Mauritania, Niger, Rwanda, and São Tomé and Prìncipe.

We identified 14 articles from six countries that examined the relation between water resources development and the prevalence of schistosomiasis in the Sahelian zone. Two cross-sectional studies, comparing mean prevalence rates in areas with and without water resources development in Mali and Ghana, both found higher rates in areas where water resources development had been implemented.

The effects of irrigation on the changing epidemiology of schistosomiasis, including the introduction of *S. mansoni* into areas previously free of this parasite, were studied in different settings of Burkina Faso, Cameroon, and Senegal. The most dramatic effect of water resources development and management on schistosomiasis in recent years was observed in northern Senegal, following the construction of the Diama barrage close to the estuary of the Senegal River in 1985, which blocked the intrusion of saltwater into the river in the dry period, and hence enabled large-scale irrigation. Pre-development prevalence rates of *Schistosoma haematobium* in children and young adults were 0-0·9% in the delta zone, 0·7-3·6% close to the river in the middle valley ("walo") and 10·4-27·2% at some distance from the river in the middle valley ("diéré").[63,64] In an irrigated area, the prevalence of *S. haematobium* was 29·7%. Meanwhile, *S. mansoni* was absent throughout the area. The first cases of *S. mansoni* in the Senegal river basin were diagnosed only 18 months after the closure of the Diama dam.[114] In 1995, the prevalence of *S. mansoni* was 4·4-43·6% in endemic villages in the delta (Figure 7.3).[65] From Lac de Guiers, situated at the margin of the delta, a prevalence of 71·8% was reported. The levels of *S. haematobium* had increased to 1·9-41·4% in endemic delta villages, 11·5% in "walo" villages and 51·6% in "diéré" villages.

Figure 7.3 Human and animal water contact in an irrigation canal in the Senegal river basin where a major outbreak of *S. mansoni* occurred after the construction of the Diama barrage in the late 1980s and early 1990s (Picture: Swiss Tropical Institute)

We identified 18 articles examining the effect of water resources development and management on schistosomiasis in tropical west and central Africa. In Côte d'Ivoire, Ghana, and Nigeria, the creation of large dam reservoirs was followed by the introduction of urinary schistosomiasis or an increase in its prevalence among residents living in close proximity to these reservoirs. The prevalence levels of intestinal schistosomiasis remained stable. For example, a study by N'Goran and colleagues[93] carried out in villages located around Lake Taabo in central Côte d'Ivoire (Figure 7.4) reported the introduction of urinary schistosomiasis into the area, reaching a prevalence of 73% in 1992, while the prevalence of intestinal schistosomiasis remained stable at about 3%.

The effect of irrigated rice farming on schistosomiasis was investigated in three studies, but no clear trend became apparent.[59,67-69]

Figure 7.4 Human exposure to schistosome-infested water at Lake Taabo, Côte d'Ivoire (Picture: Swiss Tropical Institute)

The few available studies on small reservoirs and schistosomiasis focused on *S. mansoni*. A prevalence of 21·2% was observed in an endemic area close to fish ponds, compared with a prevalence of 7·8% in more distant sites in Bafia, Cameroon (risk ratio 2·7).[86] Two studies, one from Sierra Leone[107] and one from the Democratic Republic of the Congo[108] documented the introduction of *S. mansoni* into areas previously free of the disease following mining activities. The creation of open water bodies was probably the underlying reason.

In east Africa, the effects of irrigation and small agricultural dams on schistosomiasis were assessed in eight studies done in Ethiopia, Kenya, Madagascar and Tanzania. The introduction of *S. mansoni* into the upper and middle Awash valley, Ethiopia, following the establishment of large-scale irrigation schemes has been documented for the Wonji sugar estate, where irrigation commenced in 1954 and the first case of *S. mansoni* was diagnosed one decade later. Prevalence steadily increased up to 20% in 1980[110] and in 1988, a prevalence of 81·9% was found among children of a village in the irrigation scheme.[111]

S. mansoni remained absent from the lower valley where *S. haematobium* was endemic. *S. haematobium* also did not increase in frequency.[109]

Both longitudinal and cross-sectional data are available for Ankilivalo district in western Madagascar, where a dam was built in 1979 for irrigation purposes. Although the prevalence of *S. mansoni* in school-age children rose from 13% in 1971 to 74% in 1986 in Ankilivalo, it was only 7·1% in 1986 in neighbouring Morafeno, which was not connected to the irrigation network.[60] That the irrigation system was a main trigger for schistosomiasis transmission became apparent after the destruction of the dam by a natural disaster in 1990; the prevalence in Ankilivalo decreased to 34·1% in 1994 while it had remained at 7·6% in Morafeno.61

In southern Africa, we identified four studies reporting data over 34 years for the area of Siavonga, Zambia, on the shores of Lake Kariba. A low prevalence of *S. haematobium* and the absence of intermediate host snails from the construction site had been noted in a baseline evaluation report of the medical aspects of the dam project. There was no mention of *S. mansoni* transmission.[100] The first follow-up study done 8 years after completion of the dam found prevalences of 28·8% and 5·9% for *S. haematobium* and *S. mansoni*, respectively.[99] Several studies done in the 1990s reported further raised prevalence levels - i.e., 16·7-35·5% and 33·5-60·1% for *S. haematobium* and *S. mansoni*, respectively.[101-103]

Irrigation is of paramount importance to facilitate crop production in many countries of WHO subregions 6, 7, and 9. It is estimated that 253 000 km^2 or 25% of the total arable area is currently under surface irrigation in these subregions. Three-quarters of this area is located in Egypt, Iran, Iraq, and Turkey. We estimate that 43·6 million people at risk of schistosomiasis live in irrigated areas (31.6% of the population at risk), mainly in Egypt (34 million).

Considering a 5-km belt around the reservoirs created by the 925 large dams in these subregions translates to an estimated at-risk area of 128 000 km^2, of which 82 000 km^2 are located in Turkey. An estimated 2·3 million people at risk of schistosomiasis, 1·7% of the total population at risk, live in these areas. No data on large dams are available from the World Register of Dams for Iran, Somalia, and Yemen.[17]

14 studies reporting changes in the prevalence of schistosomiasis following water resources development and management activities in Egypt and Sudan were identified. The spread of *S. mansoni* and the concurrent decline in *S. haematobium* in Egypt has been attributed to the building of the Aswan low and high dams and the subsequent intensification of irrigation. This shift was documented in a village in the delta where the prevalence of *S. haematobium* declined from 74·3% in 1935 to 2·2% in 1979, whereas the prevalence of

S. mansoni rose from 3·2% to 73% over the same time.[71] This finding could not be corroborated in a large-scale study of the whole delta region.[72]

The history of schistosomiasis in Africa's largest irrigated area, the Gezira irrigation scheme in Sudan, is well documented. From the onset of irrigation (closure of the Sennar dam in 1925), the population was regularly screened for schistosomiasis and very low levels of urinary schistosomiasis were reported up to the 1940s when an increase to 30% was noted.[76] In the same year, the prevalence of intestinal schistosomiasis was reported to be 1·3%. In the 1950s, similar infection prevalences of about 9% were reported for both parasites.[77] The construction of the Roseires dam in 1960 allowed irrigation to be further enhanced. In the 1970s and 1980s, levels of *S. mansoni* in excess of 50% were reported, while the prevalence of *S. haematobium* stood at 0-20%.[78-83] To mitigate the public health significance of intestinal schistosomiasis in the area, the "Blue Nile Health Project" was initiated in the area in 1979.[115] However, the project failed to make a dent, as the prevalence of schistosomiasis remained high.[20,83]

7.4.2.2 WHO subregion 4 (the Americas)

We estimate the area under surface irrigation of the endemic countries of this subregion to be 26 000 km^2, primarily in Brazil (17 000 km^2). This represents 3·6% of the arable land. 537 000 people at risk (1·5% of the total in this subregion) are estimated to live in those areas; 67% of them in the Dominican Republic. Of the 755 large dams constructed in WHO subregion 4, 84% are located in Brazil, as are an estimated 80% of the total area of 128 000 km^2 situated within 5 km from reservoir shores. Overall, 3·4% (1·22 million) of all people at risk of schistosomiasis live in the Americas, with most in Brazil.

Only two studies in WHO subregion 4 could be identified. The first study describes the introduction of *S. mansoni* into the Guayama/Arroyo region, Puerto Rico, following the introduction of irrigated sugar cane cultivation with peak prevalences of 40% in 6-year-old children.[113] The second study is from Bahia, Brazil, where no apparent correlation was observed between the size of the irrigated area, the prevalence of *S. mansoni*, and its historical evolution.[84]

7.4.2.3 WHO subregions 11, 12, 13 and 14 (Southeast Asia and Western Pacific)

Approximately 1 258 000 km^2 or 31% of the total arable land are currently under surface irrigation in these subregions, 90% of them in China and India. We estimate that 9·1 million

people (19·7% of the total population at risk) live in irrigated, schistosome-endemic areas, 8·76 million of them in China and 0·35 million in the Philippines. The World Register of Dams lists 10 845 large dams in the schistosome-endemic countries of these subregions. The area within 5 km of the lakeshores is estimated at 1·43 million km^2; China and India having equal shares of 0·62 million km^2 each. We further estimate that 10·1 million people live in at-risk areas or 21·7% of the total population at risk in these subregions. Virtually all of them live in China (9·97 million).

We could not identify a single study that looked at the effect of water resources development and management on schistosomiasis in these WHO subregions. This observation warrants further scrutiny - e.g., screening of the Chinese literature that is not referenced in any of the electronic databases used in the current analysis.

7.4.3 Meta-analysis

Overall, 24 studies (including 35 datasets) reported sufficiently detailed data to calculate pooled random risk ratios of schistosomiasis with regard to the construction and operation of dams and irrigation schemes. All studies stem from Africa. Heterogeneity of these studies was significant ($p < 0.001$). Risk ratio estimates for studies assessing the effect of irrigation on urinary and intestinal schistosomiasis ranged from 0·02-7·3 (summary estimate 1·1; Figure 7.5) and 0·49-23·0 (summary estimate 4·7; Figure 7.6), respectively. Pooled random risk ratios associated with dam construction were calculated as 2·4 (95% CI 1·4-3·9) and 2·6 (95% CI 1·4-5·0) for *S. haematobium* (Figure 7.7) and *S. mansoni* (Figure 7.8), respectively.

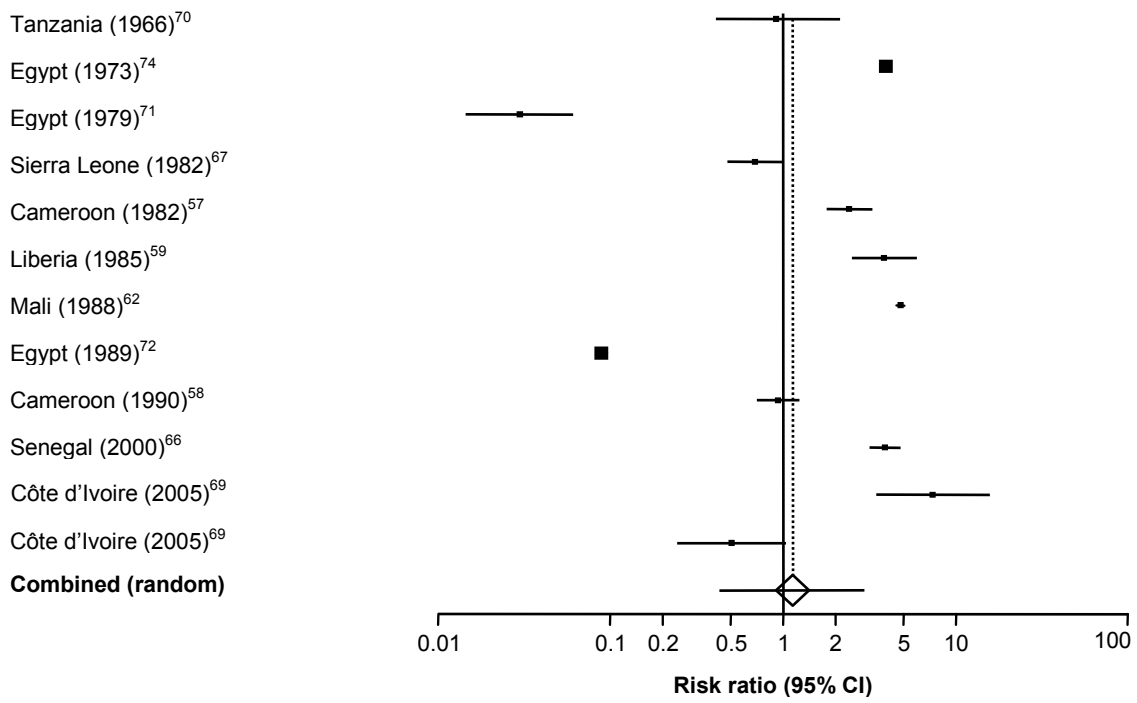

Figure 7.5 Risk ratio estimates and pooled random risk ratio of urinary schistosomiasis due to living in irrigated areas

The rectangles represent the risk ratios and the size of the rectangles represents the weight given to each study in the meta-analysis. The diamond and vertical broken line represent the combined risk ratio. The solid vertical line is the null value. Horizontal lines represent 95% CIs.

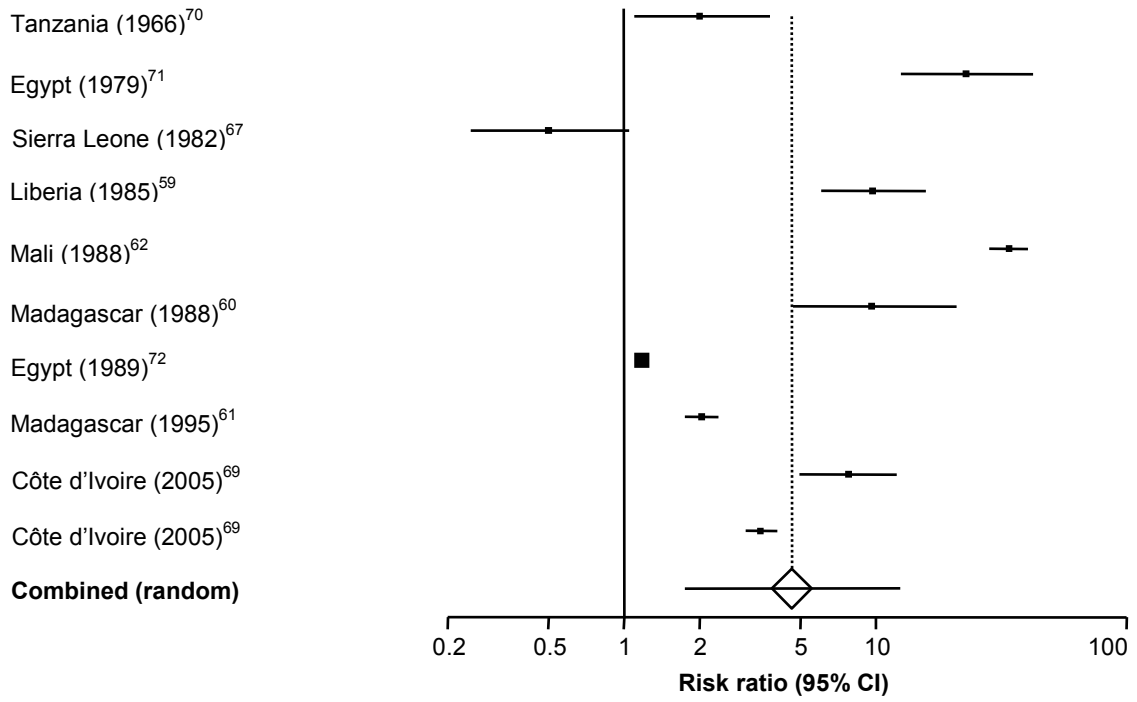

Figure 7.6 Risk ratio estimates and pooled random risk ratio of intestinal schistosomiasis due to living in irrigated areas

The rectangles represent the risk ratios and the size of the rectangles represents the weight given to each study in the meta-analysis. The diamond and vertical broken line represent the combined risk ratio. The solid vertical line is the null value. Horizontal lines represent 95% CIs.

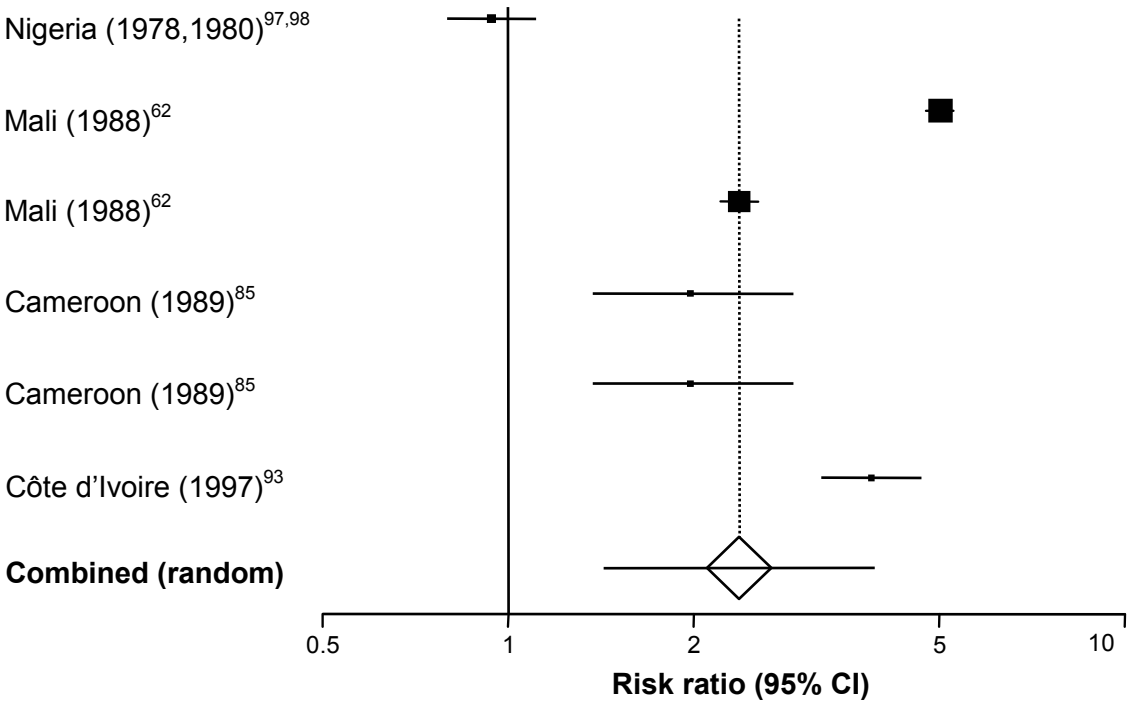

Figure 7.7 Risk ratio estimates and pooled random risk ratio of urinary schistosomiasis due to living in close proximity to dam reservoirs

The rectangles represent the risk ratios and the size of the rectangles represents the weight given to each study in the meta-analysis. The diamond and vertical broken line represent the combined risk ratio. The solid vertical line is the null value. Horizontal lines represent 95% CIs.

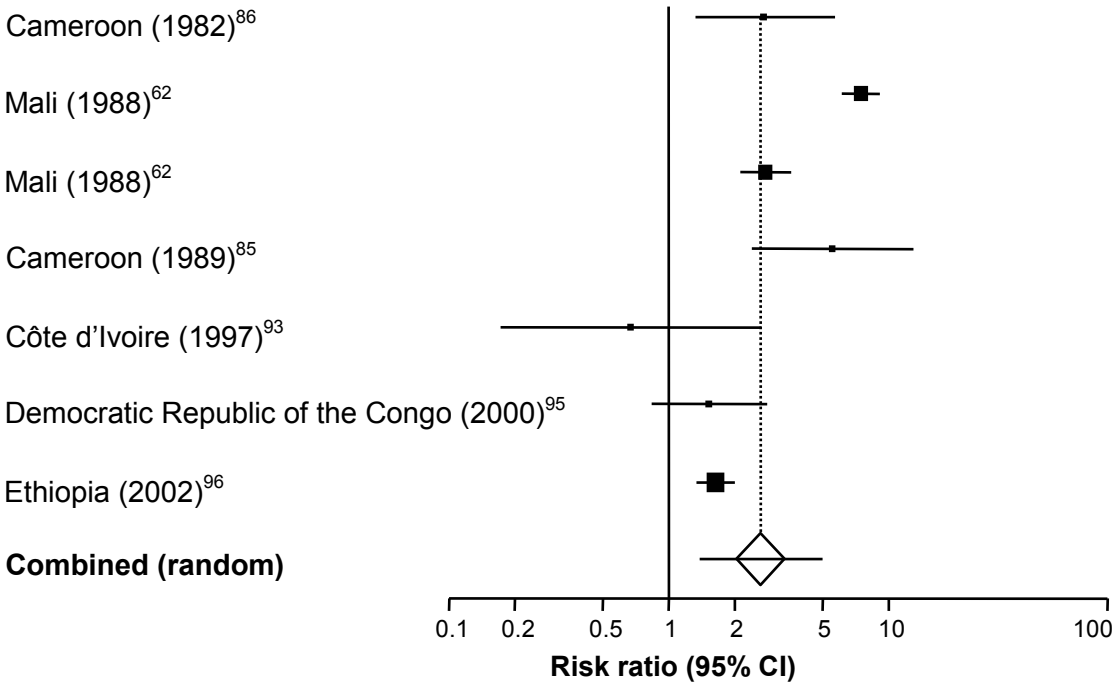

Figure 7.8 Risk ratio estimates and pooled random risk ratio of intestinal schistosomiasis due to living in close proximity to dam reservoirs

The rectangles represent the risk ratios and the size of the rectangles represents the weight given to each study in the meta-analysis. The diamond and vertical broken line represent the combined risk ratio. The solid vertical line is the null value. Horizontal lines represent 95% CIs.

7.5 Discussion

Our estimates of 779 million people at risk of schistosomiasis and 207 million infections in 2003 translate to increases of 10·9% and 7·3%, respectively, when compared with the last comprehensive estimates in the mid-1990s (at-risk population: 702 million; number of infected people: 193 million).[9] However, the relative number of people infected compared with those at risk slightly decreased from 29·6% to 26·6%. This decrease may be a consequence of wider availability and use of praziquantel and socioeconomic development (often closely linked to improved water supply and sanitation), on the one hand, and the population growth and a scarcity of new estimates of people at risk on the other. Both the increases in numbers of people infected and those at risk primarily occurred in Africa, corroborating similar observations for soil-transmitted helminthiasis; case numbers increased in Africa, while they decreased in most other regions of the world.[116]

We were able to update national schistosomiasis prevalence or incidence data for 31 countries. Unfortunately, new data are only available for six countries of WHO subregions 1 and 2 where 90% of the global cases occur. These countries are Cameroon, Mauritius, Chad, Malawi, Togo and Uganda. In the latter four countries, the reported prevalence rates were derived from surveys of school-age children. We applied these rates to the total population without further adjustment. Since school-age children usually have higher prevalence rates than younger or older population segments,[10,23,117] we are likely to overestimate the numbers of people infected in these countries. This was judged acceptable on three grounds. First, in sub-Saharan Africa the proportion of the population under 15 years of age is in the range of 40-50% in most countries.[118] Second, reported prevalence data are lower than "true prevalences" because of the lack of sensitivity of diagnostic tools.[119] Third, the total number of infections estimated for these four countries (i.e. 9 447 000) translates to only 4·6% of the global estimate.

We estimate that 106 million people at risk of schistosomiasis (13·6% of the total at-risk population) live in proximity to large dam reservoirs and irrigation schemes, about three-fifth of them are in proximity to irrigation schemes. Only areas under surface irrigation were considered because we assumed that other irrigation techniques (e.g., drip and sprinkler irrigation) pose little risk if they are well maintained.[6,120] Our estimates are primarily based on data from FAO[18,47] and the World Register of Dams.[17] At present, the number of people living close to reservoirs of small dams and on land under informal irrigation remains elusive. Ignoring small dams and informal irrigation inevitably results in an underestimate of the total number of people at risk of schistosomiasis due to water resources development. This issue

has been discussed recently with an emphasis on malaria[1] and should not be underestimated since, at least in arid and semi-arid climate zones, agriculture heavily relies on small-scale artificial irrigation.[121,122] Additional groups of people affected, but not included in our at-risk estimates, are seasonal migrants working in irrigated agricultural areas in times of increased labour demand. Their number is very large in certain irrigation schemes - e.g., in the Gezira, Sudan.[123] They can also spread the parasite to non-endemic areas given the presence of susceptible intermediate host snails.[124] The dampening effect of dams on the fluctuations of downstream water levels, which creates more stable snail habitats, was not assessed either, nor was the number of people at risk due to mining activities.

Underlying assumptions of our calculations were discussed in detail in our preceding work.[1] Additional points are offered for discussion here. We considered studies reporting pre-intervention and post-intervention data, as well as cross-sectional studies comparing settings affected by water resources development with close ecological replicates. Each type of study has some inherent limitations with regard to the generalisation of specific findings, thus also limiting the meaning of the meta-analysis. Our approximation of the rural population density overestimates the true population density in arable areas of countries with a considerable number of rural people living on land not classified as “arable” or “planted with permanent crops”. This potential overestimate is expected to correct at least partly for the often higher-than-average population density in irrigated areas[1] while still taking into account country-specific differences. The calculated mean population density in arable areas of endemic countries is 329 people per km^2 and the range of values compares well with other estimates.[1,125] The multiplication of the population living in irrigated areas and in proximity to large dam reservoirs with the national fraction at risk to derive the at-risk population almost certainly results in an underestimate, as the risk of infection in rural settings is higher than the national average. Besides, the level of irrigation and dam construction in endemic areas of a country is not necessarily proportional to the population fraction living in the respective areas. Finally, it is not always possible to clearly assign projects to either “irrigation” or “man-made lake” because many dams serve multiple purposes - e.g., flood control and provision of water for urban centres, irrigation, and hydropower generation.[122] Therefore, the distinction between the effects of dams and those of irrigation schemes is to some extent arbitrary.

An important finding of our work is that the fractions of the total population at risk of schistosomiasis, irrigated population at risk and total population at risk due to proximity to large dam reservoirs vary considerably (Table 7.1), because of major differences in contextual determinants (e.g. agricultural traditions and socioeconomic status). Only 15·8% of the

irrigated population at risk live in WHO subregions 1 and 2, reflecting the current low level of irrigation in these countries and underscoring the risk for an increase in the schistosomiasis burden once irrigation developments in Africa take off. Irrigation is much more pronounced in WHO subregion 7 where 67·4% of all people at risk due to irrigation are estimated to live. Although the number of large dams is very low in most sub-Saharan African countries, there are two notable exceptions: Zimbabwe and South Africa. Here, 38% of the global at-risk population living in proximity to large dam reservoirs are found.

It is widely acknowledged in public health circles that water resources development can amplify the risk of schistosomiasis, particularly in Africa,[19,20,23,73] but this association has also been challenged.[126] There also is a paucity of studies from other regions.[19,23,126] Whereas actual or suspected increases in the prevalence and intensity of schistosome infections repeatedly have been attributed to such activities, there are often no quantifiable baseline and follow-up data available,[127] - e.g., in Khuzestan province, Iran,[128,129] in eastern Uganda[130] and in the Kainji lake area, Nigeria.[131,132]

The summary random risk ratio of schistosomiasis due to proximity to dam reservoirs in Africa was 2·5. Although we found no raised overall risk of *S. haematobium* in irrigation schemes, the risk of intestinal schistosomiasis due to irrigation was found to be strongly correlated. This result is governed by studies documenting the so-called "Nile shift"[20] in Egypt and the inconclusive data from rice farming areas in tropical West Africa. Nevertheless, other studies showed a clear increase of urinary schistosomiasis when irrigation was initiated. The preferred habitat of the intermediate host snails offers at least some explanation for this finding. *Biomphalaria* spp. snails (intermediate host of *S. mansoni*) require more stable water levels than *Bulinus* spp. and thus are likely to benefit more from intensive irrigation.

Some interesting regional trends emerge from our analysis. First, urinary schistosomiasis often increased in populations living close to large dam reservoirs in west Africa while the introduction or spread of *S. mansoni* was associated with smaller water bodies. Second, the introduction or spread of intestinal schistosomiasis was reported in almost all studies on the effects of irrigation in east Africa. Third, both irrigation and dam reservoirs resulted in increased levels of schistosomiasis or the introduction of intestinal schistosomiasis in the Sahelian zone. Third, the introduction of *S. mansoni* into areas previously free of the parasite was observed eight times in Africa and once in the Caribbean, but the introduction of urinary schistosomiasis has been documented only twice, both times following the construction of a large dam in west Africa.

We conclude that globally, a large number of people live in areas under surface irrigation or in close proximity to large dam reservoirs, and both the irrigated area and the number of large dams are ever increasing. Our meta-analysis shows that an increase in the prevalence of schistosomiasis can result from water resources development projects. This stresses – once more – the need to include health impact assessment, including schistosomiasis risk profiling of affected populations into the screening, scoping and monitoring stages of future water projects, and to implement sound mitigation strategies.[20,133] Identified research needs include the effects of small dams and informal irrigation schemes on schistosomiasis, the interplay of water resources development and schistosomiasis in Asia (e.g., the impact of the Three Gorges dam project in China on the frequency and transmission dynamics of *Schistosoma japonicum*[134]) and the Americas and the nature of the conditions that determine why schistosomiasis is introduced into some non-endemic areas and why others remain unaffected.

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7.8 Appendix 7.1

Population at-risk of infection with *Schistosoma* spp. in endemic countries at mid-year 2003 (in thousands), stratified into WHO sub-regions (values in brackets are percentage of total population)a

WHO sub-region 1: 275 404 (86·6)

Algeria: 7441 (23·4); Angola: 13 625 (100); Benin: 6 736 (100); Burkina Faso: 13 002 (100); Cameroon: 13 706 (85·6); Chad: 6792 (79·0); Equatorial Guinea: 99 (20·0); Gabon: 1329 (100); Gambia: 1141 (80·0); Ghana: 20 922 (100); Guinea: 8480 (100); Guinea-Bissau: 1493 (100); Liberia: 2694 (80·0); Madagascar: 17 404 (100); Mali: 13 007 (100); Mauritania: 2893 (100); Mauritius: 411 (33·6); Niger: 11 972 (100); Nigeria: 112 845 (91·0); São Tomé and Prìncipe: 30 (18·5); Senegal: 10 095 (100); Sierra Leone: 4379 (88·1); Togo: 4909 (100)

WHO sub-region 2: 283 344 (77·3)

Botswana: 1785 (100); Burundi: 3033 (44·4); Central African Republic: 3865 (100); Congo: 2607 (70·0); Côte d'Ivoire: 16 631 (100); Democratic Republic of the Congo: 41 161 (78·0); Eritrea: 2197 (53·1); Ethiopia: 37 457 (53·0); Kenya: 31 987 (100); Malawi: 12 105 (100); Mozambique: 18 863 (100); Namibia: 248 (12·5); Rwanda: 5032 (60·0); South Africa: 27 916 (62·0); Swaziland: 1077 (100); Uganda: 16 700[24] (64·7)[b]; United Republic of Tanzania: 36 977 (100); Zambia: 10 812 (100); Zimbabwe: 12 891 (100)

WHO sub-region 4: 36 033 (16·5)

Antigua and Barbuda: <1 (0·5); Brazil: 28 048 (15·7); Dominican Republic: 5157 (59·0); Guadeloupe: 234 (53·1); Martinique: 69 (17·6); Montserrat: 0; Puerto Rico: 776 (20·0); Saint Lucia: 18 (12·0); Suriname: 39 (9·0); Bolivarian Republic of Venezuela: 1691[26] (6·6)[b]

WHO sub-region 6: 12629 (9·1)

Islamic Republic of Iran: 4484 (6·5); Jordan: 31 (0·6); Lebanon: 0 (0); Libyan Arab Jamahiriya: 1830 (33·0); Oman: 23 (0·8); Saudi Arabia: 4117 (17·0); Syrian Arab Republic: 1704 (9·6); Tunisia: 440 (4·5)

WHO sub-region 7: 125 344 (65·6)

Egypt: 62 597 (87·0); Iraq: 6626 (26·3); Morocco: 917 (3·0); Somalia: 4945 (50·0); Sudan: 30 249 (90·0); Yemen: 20 010 (100)

WHO sub-region 9: 72 (0·1)

Turkey: 72 (0·1)

WHO sub-region 11: 108 (<0·1)

Indonesia: 108 (<0·1); Thailand: 0 (0)

WHO sub-region 12: 13 (<0·1)

India: 13 (<0·1)

WHO sub-region 13: eliminated[25]

Japan: eliminated[25]

WHO sub-region 14: 46 371 (3·2)

Cambodia: 80[27] (0·6); China: 40 000 (3); Lao People's Democratic Republic: 60[27] (1·1); Malaysia: 0 (0); Philippines: 6231 (7·8)[c]

[a] Based on percentage at risk from Chitsulo *et al.*[9] and recalculated with figures from United Nations[46]
[b] 2002
[c] Based on Blas *et al.*[29] and recalculated with figures from United Nations[46]

7.9 Appendix 7.2

Number of people infected with *Schistosoma* spp. in endemic countries at mid-year 2003 (in thousands), stratified into WHO sub-regions (values in brackets are percentage of total population)a

WHO sub-region 1: 98 118 (30·9)

Algeria: 2385 (7·7); Angola: 6056 (44·4); Benin: 2388 (35·5); Burkina Faso: 7801 (60·0); Cameroon: 1922[30] (12·0)[b]; Chad: 1935[31] (22·5)[c]; Equatorial Guinea: 9·88 (2·0); Gabon: 604 (45·5); Gambia: 428 (30·0); Ghana: 15 172 (72·5); Guinea: 2182 (25·8); Guinea-Bissau: 448 (30·0); Liberia: 808 (24·0); Madagascar: 9579 (55·0); Mali: 7804 (60·0); Mauritania: 792 (27·4); Mauritius: 10·989[32] (0·9)[d]; Niger: 3192 (26·7); Nigeria: 28 779 (23·2); São Tomé and Prìncipe: 6·192 (3·8); Senegal: 1544 (15·3); Sierra Leone: 2959 (59·5); Togo: 1311[33] (26·7)[e]

WHO sub-region 2: 89 066 (24·3)

Botswana: 179 (10·0); Burundi: 910 (13·3); Central African Republic: 387 (10·0); Congo: 1275 (34·2); Côte d'Ivoire: 6652 (40·0); Democratic Republic of the Congo: 14 905 (28·2); Eritrea: 299 (7·2); Ethiopia: 5013 (7·1); Kenya: 7356 (23·0); Malawi: 932[34] (7·7)[f]; Mozambique: 13 158 (69·8); Namibia: 11·922 (0·6); Rwanda: 498 (5·9); South Africa: 4882 (10·8); Swaziland: 275 (25·6); Uganda: 5269[24] (20·4)[g]; United Republic of Tanzania: 19 038 (51·5); Zambia: 2871 (26·6); Zimbabwe: 5156 (40·0)

WHO sub-region 4: 1809 (0·8)

Antigua and Barbuda: 0·094 (0·1); Brazil: 1515[28,35] (0·8)[f]; Dominican Republic: 258 (2·9); Guadeloupe: 4·4[28] (1·0)[h]; Martinique: 0[26,i]; Montserrat: 0[26,j]; Puerto Rico: 4·655 (0·1)[k]; Saint Lucia: 0·059[26,l]; Suriname: 3·935 (0,9); Bolivarian Republic of Venezuela: 23·674[37] (0,1)[m]

WHO sub-region 6: 306 (0·2)

Islamic Republic of Iran: eliminated[28,38]; Jordan: 0·37[39,n]; Lebanon: eliminated[38]; Libyan Arab Jamahiriya: 278 (5·0); Oman: 0·080[40,o]; Saudi Arabia: 24·217[28] (0·1)[m]; Syrian Arab Republic: 3·787 (<0·1); Tunisia: eliminated[28,38]

WHO sub-region 7: 16 920 (8·9)

Egypt: 7193[28] (10·0); Iraq: 30 (0·1); Morocco: 0·042[41,i]; Somalia: 1780 (18·0); Sudan: 5000[38] (14·9); Yemen: 2916 (14·6)

WHO sub-region 9: 0

Turkey: eliminated[28]

WHO sub-region 11: <1

Indonesia: 0·078[42p]; Thailand: eliminated[28]

WHO sub-region 12: 0·252 (<0·1)

India: 0·252 (<0·1)

WHO sub-region 13: eliminated[25]

Japan: eliminated[25]

WHO sub-region 14: 1065 (0·1)

Cambodia: 8·0[27] (0·1)[q]; China: 800[43] (0·1); Lao People's Democratic Republic: 1·26[27] (<0·1)[q]; Malaysia: 0[44]; Philippines: (0·3)[r]

[a] Based on percentage at risk from Chitsulo *et al.*[9] and recalculated with figures from United Nations[46]
[b] 1999, data of *S. haematobium* and *S. mansoni* added
[c] 2000, prevalence in school-age children applied to whole population
[d] 1992
[e] 1997, prevalence in school-age children applied to whole population
[f] 2002, prevalence in school-age children applied to whole population
[g] 2002, prevalence of *S. mansoni* in school-age children applied to whole population
[h] 1998
[i] New cases, 2002
[j] New cases, 1997-2000
[k] Probably no new cases [26, 36]
[l] New cases, 1996-1999
[m] 1996
[n] New cases, 2000
[o] New cases, 2003
[p] New cases, 1999
[q] 2001
[r] 1996, 5881 new cases in 2001[45]

8. Helminth infections and risk factor analysis among residents in Eryuan county, Yunnan province, China

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8.1 Abstract

Whilst infections with soil-transmitted helminths are common across China, the public-health significance of *Schistosoma japonicum* and food-borne helminths is more focalized. Only few studies have investigated the local epidemiology of helminth infections in rural China, including risk factor analysis. We collected stool and blood samples from 3220 individuals, aged 5-88 years, from 35 randomly selected villages in Eryuan county, Yunnan province, China. Stool samples were subjected to the Kato-Katz technique and examined for helminth eggs. Blood samples were tested for *Trichinella* spp., *S. japonicum* and cysticerci-specific antibodies. Data on individual and family-level risk factors were collected using questionnaires. The prevalence of *Ascaris lumbricoides*, *Taenia* spp., *Trichuris trichiura* and hookworms was 15.4%, 3.5%, 1.7% and 0.3%, respectively. The seroprevalence of *Trichinella* spp. was 58.8% and that of cysticercosis 18.5%. The egg positivity rate of *S. japonicum* in the 13 known endemic villages was 2.7%, and the corresponding seroprevalence was 49.5%. We observed a strong spatial heterogeneity in the families' economic status. *S. japonicum* infections were more prevalent among the Han than Bai nationality (odds ratio (OR) = 3.77, 95% confidence interval (CI) = 1.97-7.23) and tobacco growers (OR = 3.66, 95% CI = 1.77-7.60) and was only found at elevations below 2150 m above sea level. *A. lumbricoides* and *Taenia* spp. infections were more prevalent at altitudes above 2150 m when compared to lower settings (OR = 1.51, 95% CI = 1.24-1.84 and OR = 5.32, 95% CI = 3.42-8.28, respectively). The opposite was found for *T. trichiura* (OR = 0.31, 95% CI = 0.14-0.70). Our findings can guide the design and spatial targeting of control interventions against helminth infections in Eryuan county.

Keywords: Soil-transmitted helminthiasis, schistosomiasis, *Schistosoma japonicum*, *Taenia*, *Trichinella*, cysticercosis, risk factors, multiparasitism, socio-economic status, China

8.2 Introduction

Human helminth infections are common in China, particularly the soil-transmitted helminths, namely *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (mainly *Necator americanus*) (Mao, 1991; Xu et al., 1995). Until recently, it was believed that a substantial fraction of the global burden due to soil-transmitted helminthiasis is concentrated in China (de Silva et al., 2003). However, the epidemiology of many parasitic diseases has profoundly changed in the wake of China's rapid economic development (Banister and Zhang, 2005). In fact, recent surveys found significant declines in the prevalence of several helminths. Whilst the first nationwide survey carried out in the years 1988-1992 found prevalences of 47.0%, 18.8% and 17.2% for *A. lumbricoides*, *T. trichiura* and the hookworms, respectively (Xu et al., 1995), the second survey completed in 2004 indicated a drop in the respective prevalences to 12.7%, 4.6% and 6.1% (Ministry of Health, 2005). However, China's economic development exhibits a strong spatial heterogeneity, which in turn had an impact on the frequency of soil-transmitted helminths. Whilst in the late 1980s the eastern and southern provinces were affected most by soil-transmitted helminths (Xu et al., 1995), the highest prevalences are now observed in the central and western provinces, including Yunnan (Ministry of Health, 2005).

The only human pathogenic schistosome species in China is *Schistosoma japonicum*. Today, it still occurs in the marshlands and great lakes region along the Yangtze River in eastern China, and in mountainous areas of Sichuan and Yunnan. Sustained control activities implemented over the past 50 years brought down the total number of infections by over 90% (Utzinger et al., 2005; Zhou et al., 2005). The epidemiology of schistosomiasis japonica in eastern China has been studied in considerable detail but, thus far, few studies focused on the mountainous areas in the western part of the country (Spear et al., 2004). The reasons are multifactorial, including the complex local environment with small transmission foci, remoteness and lagging socio-economic development (Chen and Feng, 1999; Ross et al., 2001).

Food-borne trematode infections (e.g. *Clonorchis sinensis*) and other food-borne helminths (e.g. *Taenia* spp., *Trichinella* spp.) have a more patchy distribution linked to certain minorities and food consumption habits such as eating raw fish and raw meat. The local prevalence of these helminth species can be high and recent data suggest that they are emerging (Hotez et al., 1997; Lun et al., 2005; Ministry of Health, 2005; Cui et al., 2006). For Yunnan province, the third nationwide sampling survey on schistosomiasis carried out in 2004 estimated a prevalence of 1.7% in the 270 schistosome-endemic villages. Meanwhile, the second national parasitological survey reported a mean prevalence of 21.7% for soil-

transmitted helminth infections (Ministry of Health, 2005). Antibodies against *Trichinella* spp. were found in 8.3% of the population; the highest rate in China.

The aim of the present study was to assess the prevalence of *S. japonicum*, soil-transmitted helminths and food-borne helminths using standardized parasitological and serological tests, and to investigate behavioural, demographic, environmental and socio-economic risk factors for infection using pre-tested questionnaires. Our geographical focus is on a partially schistosome-endemic county in Yunnan province.

8.3 Materials and methods

8.3.1 Study area and sample size

Eryuan county is situated in Dali prefecture, in the north-west of Yunnan province in southern China (25.80°-26.43° N latitude, 99.54°-100.34° E longitude, ~3000 km^2). The county features two distinct landscapes: (i) fertile plains at an elevation of 1950-2150 m above sea level between Eryuan Lake and Erhai Lake; and (ii) mountain ranges with peaks of over 3000 m in the other regions. The mountain slopes are covered by fields or coniferous forests with a tree line at ~3000 m. The total population of Eryuan county was 273,000 in 2005; the majority of them belonging to the Bai ethnic group. Both Bai and the immigrated Han are primarily engaged in agriculture and animal husbandry. The intended sample size was 1% of the local residents, i.e. about 2700 people.

8.3.2 Population surveyed, informed consent and treatment

A 3.5 x 3.5 km-grid was laid over a map of the study area and 35 squares were randomly selected among the populated grid cells (Gyapong and Remme, 2001). If the cell contained two or more natural villages, one was randomly selected among those with at least 35 families. Subsequently, 35 families per community were randomly selected from the available village registries. Natural villages are spatial accumulations of houses within an administrative village; the lowest administrative level in China. All family members aged ≥5 years were included.

The study was approved by the institutional review boards of the Swiss Tropical Institute (Basel, Switzerland) and the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, China). The leaders of the selected communities were invited to Eryuan city and informed about the purpose and procedures of the study in the presence of leading administrative, medical and veterinary county officials. The objective,

procedures, potential risks and benefits were explained and the village leaders were asked for their consent and support to conduct the study. Written informed consent was obtained from the heads of participating households. Those people tested positive for parasites in their stool were treated free of charge with appropriate drugs (i.e. praziquantel and/or albendazole) according to local and national standard treatment protocols (Li and Guan, 2004). Seropositives were informed and further testing was recommended.

8.3.3 Field procedures and questionnaires

The fieldwork was carried out in November/December 2005. Firstly, the selected families were contacted by a senior member of our research team. In case the head of household was absent or refused to participate, the neighbouring household was invited to participate. Secondly, a household questionnaire was administered to the head of the household. This questionnaire assessed house characteristics (e.g. building type and water supply), assets owned (e.g. TV and bicycle), and ownership of land and animals. Third, individual questionnaires were administered to all study participants aged ≥15 years. Younger participants answered the questionnaire with the assistance of their parents or legal guardians. Demographic information and data on raw food consumption and hand washing behaviour were collected. Both questionnaires were pre-tested in the study area with people who did not otherwise participate in the survey.

Fourth, the geographical coordinates of the participating households were recorded, using a hand-held global positioning system (GPS) receiver (Garmin Ltd.;Olathe, USA). Finally, a venous blood sample of 3-5 ml was collected and labelled plastic bags for stool collection were handed out. Stool samples were either collected by fieldworkers or by a designated resident and sent to the local schistosomiasis control station in Eryuan city where they were kept in a cool place pending analysis.

8.3.4 Laboratory procedures

Four 42 mg Kato-Katz thick smears were prepared from each stool sample (Katz et al., 1972) and read by experienced technicians. The number of eggs of each parasite species was counted and recorded separately. A sub-sample of 5% of the slides was re-examined by the senior technician for quality control.

The serum samples were kept frozen and tested by enzyme-linked immunosorbent assay (ELISA) for IgG antibodies against *S. japonicum* (Shenzhen Combined Biotech Co. Ltd.;

Shenzhen, China), cysticerci (larval stage of *T. solium*) and *Trichinella* spp. (both Hai Tai Co. Ltd; Zhuhai, China), according to the manufacturer's instructions.

The sensitivity of the approach taken to detect *S. japonicum*-infections was assessed by collecting up to three stool specimens from all participants in three *S. japonicum*-endemic villages, which were then subjected to the Kato-Katz technique plus the hatching test (Justesen and van Sloterdijck, 1977).

8.3.5 Data management and statistical analysis

The questionnaire data were double entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). All further analyses were carried out in STATA version 9 (StataCorp LP; College Station, USA). The socio-economic status of the families was calculated according to an asset-based method put forward by HNP/World Bank (Gwatkin et al., 2000) and described in detail by Raso et al. (2005). In brief, asset weights were defined by principal component analysis (PCA) after replacement of missing values by the mean of the respective asset. With the exception of the number of cows and the area of irrigated agricultural land (measured in mǔ, a traditional Chinese surface unit; 1 mǔ = 666.7 m^2), all assets had dichotomous character. The families were ranked into wealth quintiles according to their cumulative standardized asset scores.

The χ^2-test was used to explore associations between the infection status and age and sex. Bivariate logistic regression was used to test for associations between infection and demographic variables, socio-economic status, village location, health-related behaviour and raw food consumption. Associations of infection status with hand washing behaviour and raw food consumption were further assessed by multiple logistic regression analysis. The models were adjusted for demographic variables, socio-economic status and village location whenever necessary (i.e. $P < 0.1$ in bivariate analysis). Non-predicting covariates were removed at a level of $P = 0.15$, using a stepwise backward elimination procedure.

The final analysis only considered participants with complete questionnaire, parasitological and serological data. The family-level calculations were based on all available questionnaires, irrespective of stool and blood sample submission.

8.4 Results

8.4.1 Study cohort, demographic and socio-economic profile

Of 5402 registered individuals in 1225 families selected for the study, 3791 people from 1222 families were present during our cross-sectional survey and answered the questionnaire (Figure 8.1). Among them, 3617 (95.4%) consent to blood sampling and 3314 (87.4%) submitted a stool sample. Complete questionnaire data, blood and stool samples were available for 3220 individuals (84.9%). Compliance for stool and blood sample provision varied between 45.6% and 96.6% among interviewed individuals at the unit of the village.

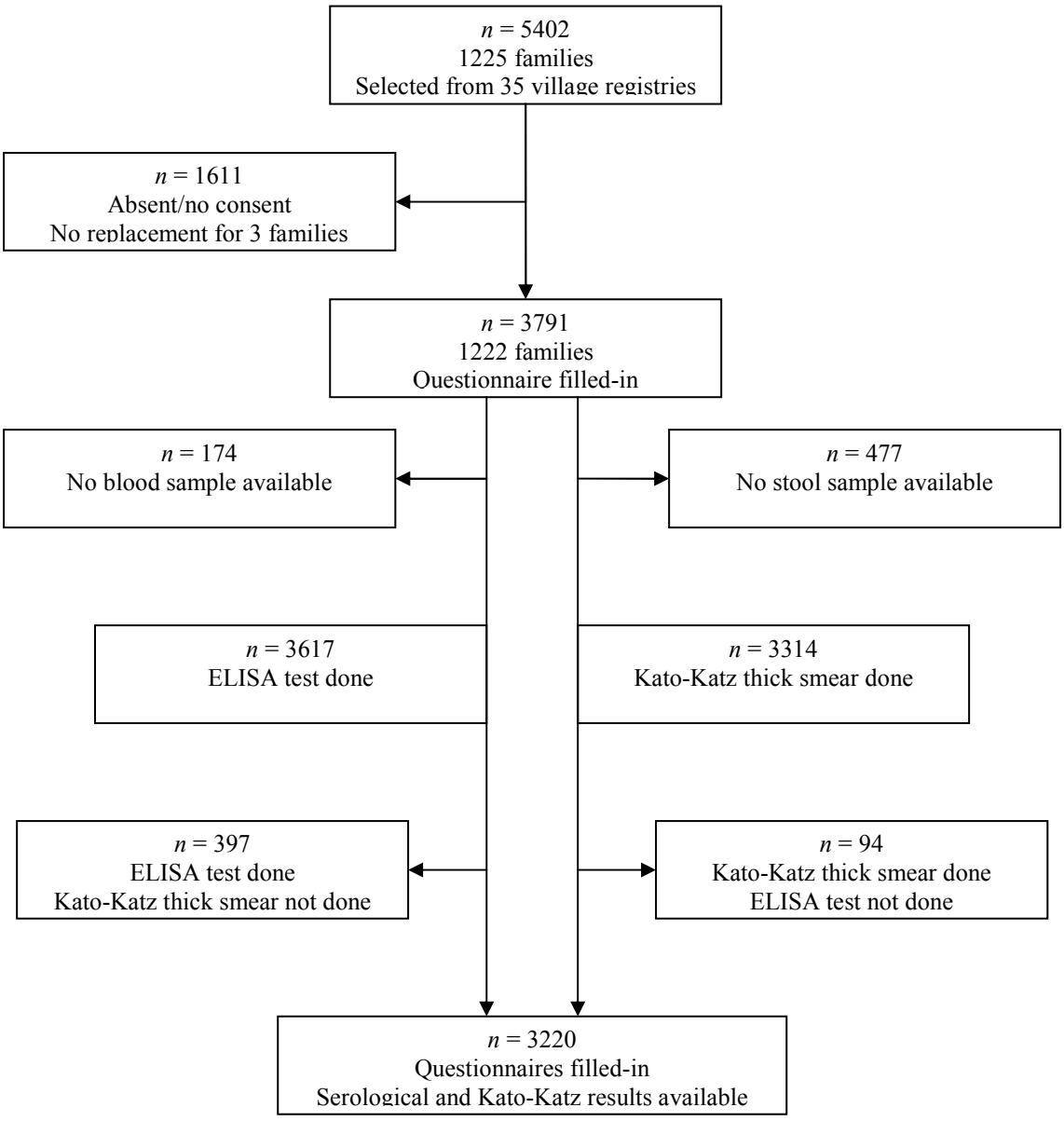

Figure 8.1 Diagram detailing the study participation and compliance with blood and stool sample submission and being interviewed in 35 randomly selected villages in Eryuan county, Yunnan province, China. The final cohort comprised those with complete questionnaire, parasitological and serological data.

The study included 13 known schistosome-endemic villages that were home to 1429 individuals who had complete data records (44.4%). According to a local classification of village location, there were nine "plateau" villages, inhabited by 25.8% of the families, and 26 "mountain" villages, situated at 1750-2700 m. Most of the families (88.3%) lived in a 2-storey house with adobe walls and wooden load carrying parts.

From our final study cohort, 55.7% were females. The age structure was as follows: 5-9 years (8.6%), 10-14 years (7.7%), 15-24 years (12.9%), 25-39 years (35.0%) and ≥40 years (35.8%). There was no statistically significant difference in the number of males and females in the different age groups ($\chi^2 = 5.83$, degree of freedom (d.f.) = 4, $P = 0.212$). Bai was the dominant ethnic group (80.3%) and Han Chinese accounted for the remaining 19.7%. While Bai lived in plain and mountainous regions, Han predominantly lived in the fertile and economically more advanced plain areas. Most participants were farmers (79.7%) or students (15.8%). Only 5.0% reported significant non-agricultural sources of income. Domestic animals were kept by 95.9% of the families. Pigs and dogs were particularly common (78.4% and 67.6%, respectively). Cattle were owned by 37.6% and cows by 26.8% of the families, the former only in mountain villages and the latter predominantly in plain areas. Other animals included goats (19.5%), mules (16.7%), horses (9.2%), buffaloes (9.2%), sheep (4.6%) and donkeys (2.0%).

The first principal component of the model used to assess the socio-economic status of the families explained 29.7% of the total variability. As shown in Table 8.1, the greatest weights were attached to households possessing a bicycle (0.35) or an electric rice cooker (0.34). Owning a black/white TV had the lowest weight (0.09). Standardization of the asset weights resulted in greatest weights being attached to cars (0.90) and motorbikes (0.89). Lowest scores resulted from the absence of a colour TV (-0.33). Every mǔ of irrigated land or cow increased the score of a household by 0.32 or 0.14, respectively. None of the assets investigated were owned by the poorest families.

Common assets were irrigated land (62.0%) and a colour TV (54.5%). Assets owned by more than 90% of the least poor families included irrigated land or a colour TV (both 97.1%), and an electric rice cooker (90.2%). Among the very poor households, 58% owned irrigated land, 19.0% a black/white TV and 13.9% a radio. Only 9.9% of the households were not connected to the power grid, but in the lowest quintile, electricity was not available in 39.9% of the households (data not presented).

Table 8.1 Household assets considered for the calculation of the socio-economic status of the families, their factor scores, score of household if asset present or absent and asset distribution among families, stratified into 5 wealth quintiles

Asset	Asset factor score	Household score asset present	Household score asset absent	No.	Household have asset *n* (%)	1st quintile Most poor	2nd quintile Very poor	3rd quintile Poor	4th quintile Less poor	5th quintile Least poor
						n (%) [total = 253]	*n* (%) [total = 237]	*n* (%) [total = 244]	*n* (%) [total = 244]	*n* (%) [total = 244]
Telephone	0.309	0.60	-0.16		257 (21.0)	0 (0)	2 (0.8)	23 (9.4)	60 (24.6)	172 (70.5)
Radio	0.157	0.37	-0.07		183 (15.0)	0 (0)	33 (13.9)	24 (9.8)	38 (15.6)	88 (36.1)
Black/white TV	0.087	0.18	-0.04		237 (19.4)	0 (0)	45 (19.0)	62 (25.4)	62 (25.4)	68 (27.8)
Colour TV	0.305	0.28	-0.33		666 (54.5)	0 (0)	67 (28.3)	163 (66.8)	199 (81.6)	237 (97.1)
VCD player[a]	0.308	0.45	-0.21		393 (32.2)	0 (0)	1 (0.4)	81 (33.2)	114 (46.7)	197 (80.7)
Electric fan	0.203	0.53	-0.08		156 (12.8)	0 (0)	3 (1.3)	17 (7.0)	54 (22.1)	82 (33.6)
Electric rice cooker	0.336	0.42	-0.27		471 (38.5)	0 (0)	3 (1.3)	80 (32.8)	168 (68.9)	220 (90.2)
Washing machine	0.318	0.71	-0.14		204 (16.7)	0 (0)	0 (0)	1 (0.4)	46 (18.9)	157 (64.3)
Refrigerator	0.236	0.86	-0.06		85 (7.0)	0 (0)	0 (0)	0 (0)	13 (5.3)	72 (29.5)
Bicycle	0.345	0.53	-0.23		367 (30.0)	0 (0)	0 (0)	31 (12.7)	130 (53.3)	206 (84.3)
Motorbike	0.238	0.89	-0.06		81 (6.6)	0 (0)	0 (0)	2 (0.8)	7 (2.9)	72 (29.5)
Tractor	0.192	0.70	-0.05		85 (7.0)	0 (0)	0 (0)	5 (2.1)	14 (5.7)	66 (27.1)
Car	0.100	0.90	-0.01		15 (1.2)	0 (0)	0 (0)	2 (0.8)	1 (0.4)	12 (4.9)
No. of cows owned	0.289	1: 0.17	-0.16	0	894 (72.2)	253 (100)	228 (96.2)	203 (83.2)	135 (55.3)	75 (30.7)
		+ 0.32/cow		1	149 (12.2)	0 (0)	9 (3.8)	23 (9.4)	63 (25.8)	54 (22.1)
				≥2	179 (14.6)	0 (0)	0 (0)	18 (7.4)	46 (18.9)	115 (47.1)
Mǔ irrigated land for agriculture[b]	0.259	1: -0.09	-0.23	0	464 (38.0)	253 (100)	99 (41.8)	81 (33.2)	24 (9.8)	7 (2.9)
		+ 0.14/mǔ		0.1-1.9	255 (20.9)	0 (0)	79 (33.3)	58 (23.8)	65 (26.6)	53 (21.7)
				2.0-3.9	350 (28.7)	0 (0)	50 (21.1)	76 (31.2)	105 (43.0)	119 (48.8)
				≥4.0	153 (12.4)	0 (0)	9 (3.8)	29 (11.9)	50 (20.5)	65 (26.6)

[a] VCD: video compact disc
[b] 1 mǔ = 666.7 m^2

8.4.2 Helminth infections

The results from our cross-sectional parasitological and serological survey, stratified by sex and age, are summarized in Table 8.2. The overall prevalence of *A. lumbricoides* infection was 15.4% and showed large village-to-village variation (0.8-48.0%). The prevalence of *Taenia solium/T. saginata* infection was 3.5% (range: 0-19.1%), that of *T. trichiura* 1.7% (range: 0-26.7%) and that of hookworms 0.3% (only found in 9 villages). *S. japonicum* eggs were found in 1.3% of all participants. The prevalence of *S. japonicum* in the 13 known endemic villages was 2.7% (range: 0-11.6%). No statistically significant difference was observed in the prevalence of any parasite by either sex or age ($P > 0.05$). Overall, helminth eggs were found in the stools of 20.5% of all participants; 18.8% of the cohort was infected by a single species, whereas dual (1.6%) and triple infections (0.1%) were rare.

Antibodies against *Trichinella* spp. and cysticerci were found in 58.8% and 18.5% of the blood samples, respectively, and the schistosomiasis seroprevalence in the 13 known endemic villages was 49.5%. The seroprevalence of trichinellosis was significantly higher in males than in females ($P < 0.001$), and increased with age ($P < 0.001$). The schistosomiasis seroprevalence was higher in females when compared to males ($P = 0.002$) and reached the highest level in the 25-39 year age group ($P < 0.001$).

Significant associations were found between infections with different parasites or serostatus. *A. lumbricoides* infection was associated with *T. trichiura* (odds ratio (OR) = 4.03, $P < 0.001$), trichinellosis seropositivity (OR = 0.64, $P < 0.001$) and *S. japonicum* seropositivity among all study participants (OR = 0.54, $P < 0.001$). *Taenia* spp. eggs were found more often in the stools of those with positive cysticercosis serology (OR = 4.59, $P < 0.001$), but were less frequent among *S. japonicum* seropositives in the whole study cohort (OR = 0.49, $P = 0.007$). Eggs of *S. japonicum* were found more often among cysticercosis seropositives in schistosome-endemic villages (OR = 2.14, $P = 0.043$). Trichinellosis seropositivity was associated with the detection of *S. japonicum* eggs and antibodies in the whole study area and in *S. japonicum*-endemic villages only (OR = 4.21-9.30).

Table 8.2 Prevalence of parasitic infections diagnosed by the Kato-Katz technique and seroprevalence assessed by ELISA in 35 randomly selected villages in Eryuan county, Yunnan province, China. Data are stratified by sex and age

Parasite	Prevalence (95% CI)	Sex				Age (years)						
		F	M	χ^2	*P*-value	5-9	10-14	15-24	25-39	≥40	χ^2	*P*-value
Eggs detected												
Ascaris lumbricoides[a]	15.4 (14.2-16.7)	16.3	14.3	2.41	0.120	19.9	18.6	14.4	14.6	14.8	7.54	0.110
Taenia spp.[a]	3.5 (2.9-4.2)	3.2	4.0	1.55	0.214	1.5	3.6	3.8	4.0	3.5	4.35	0.360
Trichuris trichiura[a]	1.7 (1.2-2.1)	1.6	1.7	0.02	0.886	1.1	3.2	1.2	1.1	2.2	9.19	0.056
Schistosoma japonicum[a]	1.3 (0.9-1.8)	1.4	1.2	0.25	0.614	0.7	2.8	0.7	1.2	1.5	6.77	0.149
Schistosoma japonicum[b]	2.7 (1.9-3.6)	2.7	2.7	<0.01	0.984	1.7	6.2	1.9	2.4	2.6	6.45	0.168
Infection with any helminth[a]	20.5 (19.1-21.9)	21.1	19.8	0.850	0.357	22.1	26.3	18.7	19.7	20.3	6.85	0.144
Antibodies detected												
Schistosoma japonicum ELISA[a]	27.1 (25.6-28.7)	29.2	24.5	8.93	0.003	11.2	20.7	22.3	31.8	29.4	60.85	<0.001
Schistosoma japonicum ELISA[b]	49.5 (46.9-52.1)	53.2	44.9	8.47	0.002	22.3	40.7	48.2	58.0	50.0	53.68	<0.001
Cysticercosis ELISA[a]	18.5 (17.2-19.9)	17.1	20.3	7.13	0.018	13.8	18.2	17.5	19.2	19.4	5.37	0.252
Trichinella spp. ELISA[a]	58.8 (57.0-60.5)	55.4	63.0	24.48	<0.001	24.6	44.1	48.0	63.5	69.3	238.07	<0.001

[a] All villages (n = 3220 individuals)
[b] In the 13 known *S. japonicum*-endemic villages (n = 1429 individuals)

8.4.3 Ethnicity, educational attainment and occupation

Table 8.3 and Table 8.4 show that among the participants aged 18 years and above, illiterates were more likely to be infected with *A. lumbricoides* than those with basic education (OR = 0.76, $P = 0.023$) or higher education (OR = 0.47, $P = 0.027$). *S. japonicum* infection, as assessed by the Kato-Katz technique, was more prevalent among Han than Bai (5.8% *versus* 1.6%; OR = 3.77, $P < 0.001$). The former ethnic group was also more likely to have antibodies against this parasite (OR = 2.40, $P < 0.001$), as well as against *Trichinella* spp. (OR = 2.51, $P < 0.001$). Tobacco growing was another risk factor for *S. japonicum* infection (7.0% *versus* 2.0%; OR = 3.66, $P < 0.001$) and seropositivity (OR = 4.32, $P < 0.001$). In addition, growing tobacco was positively associated with cysticercosis (OR = 1.57, $P < 0.001$) and trichinellosis seropositivity (OR = 1.98, $P < 0.001$).

8.4.4 Socio-economy, environment and behaviour

Eryuan county is geographically and economically heterogeneous. The spatial variation of the socio-economic composition in the random sample of villages considered here is depicted in Figure 8.2. The proportion of lower strata was higher in the western mountains. Only 1.8% of the poorest and 96.6% of the least poor families lived at an altitude <2150 m. None of the poorest and only 1.7% of the very poor families lived in plateau villages where 69.3% of the least poor families lived. Families of all five socio-economic strata were found in only five villages, and all families of one village fell into the 'most poor' category. Members of poor, less poor and least poor families were significantly less likely to be infected with *A. lumbricoides* or *Taenia* spp. than the poorest (Table 8.3). Table 8.4 shows that in known schistosome-endemic villages, members of less and least poor families were less likely to be *S. japonicum* seropositive (OR = 0.54, $P = 0.004$ and OR = 0.48, $P = 0.001$, respectively). The lowest *Trichinella* spp. antibody seroprevalence was found among the most poor.

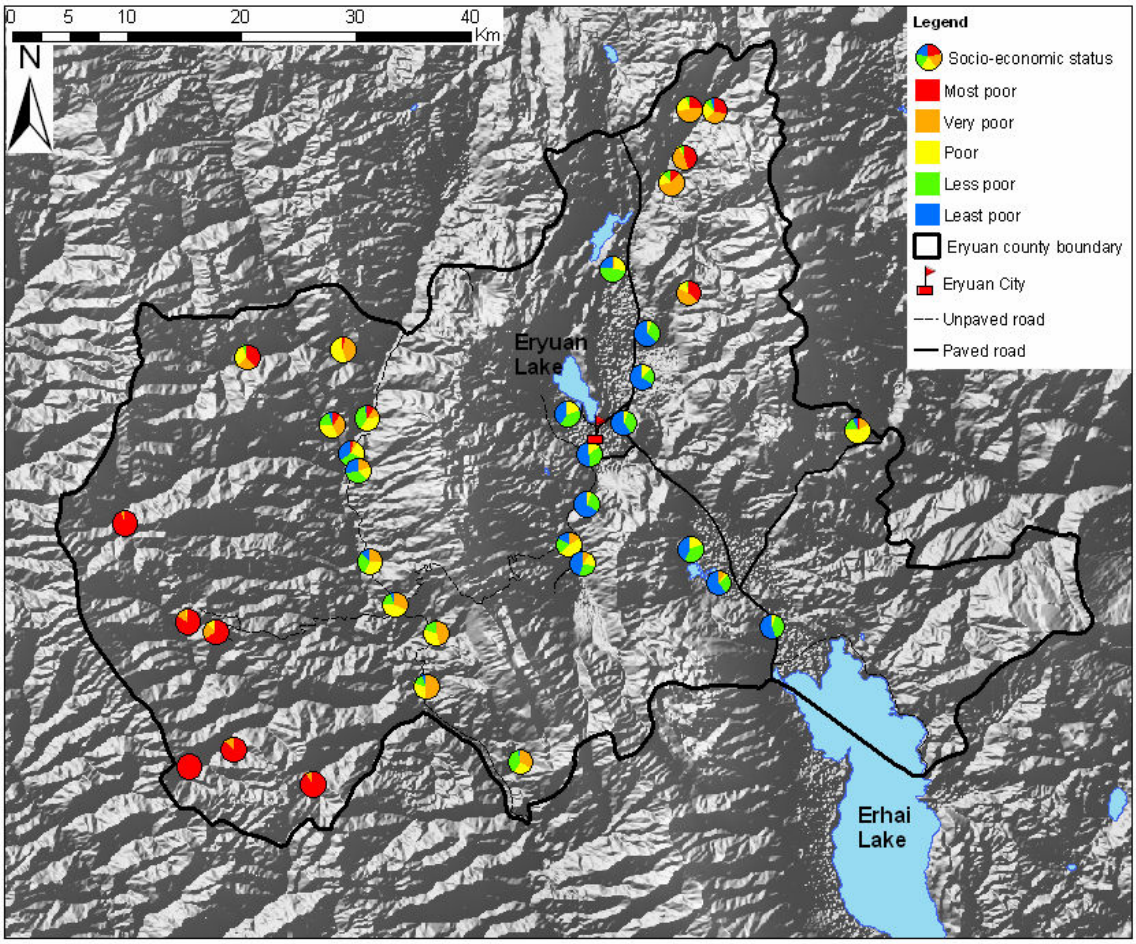

Figure 8.2 Physical relief of Eryuan county, Yunnan province, China, based on data downloaded from the Shuttle Radar Topography Mission, U.S. Geological Survey website (http://www.usgs.gov/). The location of the study villages and the respective wealth quintile distribution among the participating families are depicted. Also shown is the road network.

Table 8.3 Bivariate logistic regression analyses of the relationship between the infection risk with different helminths and demographic indicators, hand washing behaviour, raw food consumption, village location and socio-economic status in Eryuan county, Yunnan province, China

Risk factor	*Ascaris lumbricoides*[a]		*Taenia* spp.[a]		*Trichuris trichiura*[a]		*Schistosoma japonicum*[b]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sex								
Female	1.00		1.00		1.00		1.00	
Male	0.86 (0.71-1.04)	0.120	1.27 (0.87-1.84)	0.215	1.04 (0.60-1.80)	0.886	1.01 (0.53-1.92)	0.984
Age (years)								
5-9	1.00		1.00		1.00		1.00	
10-14	0.92 (0.59-1.42)	0.706	2.57 (0.78-8.46)	0.120	3.05 (0.80-11.61)	0.103	3.93 (0.80-19.33)	0.092
15-24	0.68 (0.45-1.01)	0.056	2.71 (0.90-8.20)	0.077	1.10 (0.26-4.66)	0.892	1.12 (0.18-6.82)	0.090
25-39	0.69 (0.49-0.96)	0.029	2.83 (1.01-7.94)	0.048	0.98 (0.27-3.50)	0.975	1.48 (0.33-6.69)	0.612
≥40	0.70 (0.50-0.98)	0.037	2.44 (0.87-6.88)	0.091	2.02 (0.60-6.72)	0.254	1.59 (0.36-7.09)	0.543
Education[c]								
Illiterate	1.00		1.00		1.00		1.00	
≤Junior middle school	0.76 (0.61-0.96)	0.023	0.71 (0.47-1.08)	0.110	1.20 (0.60-2.36)	0.607	0.95 (0.35-2.58)	0.925
≥High middle school	0.47 (0.24-0.92)	0.027	0.18 (0.02-1.32)	0.091	n.a.	n.a.	2.04 (0.47-8.76)	0.340
Ethnic group								
Bai	1.00		1.00		1.00		1.00	
Han	0.93 (0.73-1.19)	0.570	0.82 (0.49-1.34)	0.423	0.95 (0.48-1.91)	0.893	3.77 (1.97-7.23)	<0.001
Socio-economic status								
Most poor	1.00		1.00		1.00		1.00	
Very poor	0.80 (0.59-1.08)	0.143	0.78 (0.48-1.27)	0.323	3.73 (1.05-13.29)	0.042	n.a.	n.a.
Poor	0.61 (0.45-0.82)	0.001	0.55 (0.33-0.93)	0.024	4.28 (1.23-14.86)	0.022	0.72 (0.16-3.29)	0.674
Less poor	0.56 (0.41-0.75)	<0.001	0.27 (0.14-0.50)	<0.001	3.00 (0.84-10.70)	0.090	0.86 (0.24-3.13)	0.817
Least poor	0.51 (0.38-0.68)	<0.001	0.10 (0.04-0.24)	<0.001	2.44 (0.68-8.78)	0.173	1.21 (0.35-4.13)	0.765
Livestock breeder	0.54 (0.44-0.67)	<0.001	0.90 (0.61-1.32)	0.573	0.46 (0.25-0.87)	0.017	0.76 (0.39-1.46)	0.406
Tobacco grower	0.65 (0.45-0.93)	0.019	1.24 (0.70-2.20)	0.454	0.53 (0.16-1.71)	0.288	3.66 (1.77-7.60)	<0.001
Temporary employment	1.65 (0.98-2.78)	0.060	1.39 (0.50-3.87)	0.526	1.51 (0.36-6.31)	0.573	n.a.	n.a.

Risk factor	*Ascaris lumbricoides*[a]		*Taenia* spp.[a]		*Trichuris trichiura*[a]		*Schistosoma japonicum*[b]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Resident at altitude ≥2150 m	1.51 (1.24-1.84)	<0.001	5.32 (3.42-8.28)	<0.001	0.31 (0.14-0.70)	0.005	n.a.	n.a.
Village location								
Plain area	1.00		1.00		1.00		1.00	
Mountains	1.43 (1.16-1.77)	0.001	5.39 (2.80-10.35)	<0.001	1.15 (0.64-2.08)	0.636	0.73 (0.35-1.52)	0.403
Washing hands								
Before meals	0.89 (0.75-1.07)	0.227	0.45 (0.29-0.70)	<0.001	1.09 (0.72-1.65)	0.677	0.83 (0.44-1.59)	0.581
After defecation	0.95 (0.82-1.11)	0.517	0.49 (0.32-0.75)	0.001	1.05 (0.73-1.52)	0.795	0.71 (0.37-1.34)	0.285
Food consumption								
Raw pork	0.52 (0.40-0.69)	<0.001	0.86 (0.48-1.55)	0.611	0.27 (0.15-0.50)	<0.001	3.17 (0.43-23.37)	0.257
Raw beef	0.84 (0.67-1.05)	0.122	0.46 (0.27-0.79)	0.005	1.81 (1.03-3.18)	0.039	0.50 (0.23-1.11)	0.088
Raw fish	1.37 (0.76-2.48)	0.294	0.38 (0.05-2.79)	0.343	0.86 (0.12-6.31)	0.881	3.22 (0.73-14.16)	0.122
Raw vegetables	0.84 (0.52-1.34)	0.456	2.29 (0.56-9.40)	0.248	0.48 (0.17-1.36)	0.169	1.82 (0.24-13.44)	0.559
Raw water plants	0.77 (0.64-0.93)	0.008	0.40 (0.27-0.60)	<0.001	0.66 (0.38-1.14)	0.138	0.56 (0.29-1.07)	0.078

OR: odds ratio, CI: confidence interval, *P*: based on likelihood ratio test, n.a.: not applicable
[a] All villages (n = 3220 individuals)
[b] In the 13 known *S. japonicum*-endemic villages (n = 1429 individuals)
[c] Among participants aged ≥18 years (n = 2549; n = 1131 in the 13 known *S. japonicum*-endemic villages)

Table 8.4 Bivariate logistic regression analyses of the relationship between the serostatus and demographic indicators, hand washing behaviour, raw food consumption, village location and socio-economic status in Eryuan county, Yunnan province, China

Risk factor	*Schistosoma japonicum* ELISA[a]		Cysticercosis ELISA[b]		*Trichinella* spp. ELISA[b]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sex						
Female	1.00		1.00		1.00	
Male	0.72 (0.58-0.89)	0.002	1.24 (1.04-1.48)	0.018	1.37 (1.19-1.58)	<0.001
Age (years)						
5-9	1.00		1.00		1.00	
10-14	2.39 (1.35-4.22)	0.003	1.40 (0.87-2.23)	0.166	2.42 (1.67-3.50)	<0.001
15-24	3.23 (1.91-5.48)	<0.001	1.33 (0.87-2.03)	0.190	2.82 (2.02-3.94)	<0.001
25-39	4.80 (3.02-7.64)	<0.001	1.49 (1.02-2.16)	0.037	5.32 (3.94-7.18)	<0.001
≥40	3.48 (2.20-5.52)	<0.001	1.51 (1.04-2.19)	0.030	6.91 (5.11-9.34)	<0.001
Education[c]						
Illiterate	1.00		1.00		1.00	
≤Junior middle school	1.10 (0.81-1.49)	0.533	0.71 (0.58-0.88)	0.001	1.96 (1.64-2.33)	<0.001
≥High middle school	0.65 (0.37-1.15)	0.137	0.55 (0.32-0.97)	0.037	2.37 (1.53-3.68)	<0.001
Ethnic group						
Bai	1.00		1.00		1.00	
Han	2.40 (1.87-3.07)	<0.001	1.07 (0.86-1.34)	0.536	2.51 (2.06-3.05)	<0.001
Socio-economic status						
Most poor	1.00		1.00		1.00	
Very poor	n.a.		0.98 (0.73-1.32)	0.913	2.48 (1.94-3.17)	<0.001
Poor	0.71 (0.44-1.14)	0.158	1.20 (0.91-1.60)	0.197	2.34 (1.84-2.97)	<0.001
Less poor	0.54 (0.35-0.82)	0.004	0.82 (0.61-1.09)	0.168	3.77 (2.97-4.78)	<0.001
Least poor	0.48 (0.32-0.73)	0.001	0.63 (0.47-0.84)	0.002	4.70 (3.71-5.96)	<0.001
Livestock breeder	1.20 (0.97-1.48)	0.090	1.02 (0.85-1.23)	0.802	1.89 (1.63-2.19)	<0.001
Tobacco grower	4.32 (2.91-6.41)	<0.001	1.57 (1.20-2.05)	0.001	1.98 (1.53-2.55)	<0.001
Temporary employment	1.44 (0.63-3.26)	0.383	1.22 (0.72-2.08)	0.453	1.06 (0.68-1.66)	0.788

Risk factor	*Schistosoma japonicum* ELISA[a]		Cysticercosis ELISA[b]		*Trichinella* spp. ELISA[b]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Resident at altitude ≥2150 m	n.a.		1.63 (1.36-1.96)	<0.001	0.47 (0.41-0.55)	<0.001
Village location						
Plain area	1.00		1.00		1.00	
Mountains	1.02 (0.82-1.27)	0.870	1.73 (1.41-2.12)	<0.001	0.37 (0.32-0.43)	<0.001
Washing hands						
Before meals	0.88 (0.72-1.09)	0.239	0.78 (0.65-0.93)	0.006	1.32 (1.15-1.52)	<0.001
After defecation	1.01 (0.86-1.19)	0.926	0.75 (0.63-0.89)	0.001	1.18 (1.05-1.33)	0.006
Food consumption						
Raw pork	1.25 (0.85-1.84)	0.261	1.47 (1.06-2.05)	0.023	1.38 (1.09-1.74)	0.007
Raw beef	0.77 (0.62-0.92)	0.088	0.92 (0.74-1.13)	0.409	1.49 (1.26-1.76)	<0.001
Raw fish	1.20 (0.55-2.61)	0.647	0.90 (0.48-1.68)	0.731	1.82 (1.08-3.07)	0.025
Raw vegetables	1.44 (0.87-2.37)	0.158	1.27 (0.77-2.08)	0.352	0.67 (0.46-0.98)	0.040
Raw water plants	0.86 (0.70-1.07)	0.180	0.93 (0.78-1.11)	0.445	1.09 (0.95-1.25)	0.235

OR: odds ratio, CI: confidence interval, *P*: based on likelihood ratio test, n.a.: not applicable
[a] In the 13 known *S. japonicum*-endemic villages (n = 1429 individuals
[b] All villages (n = 3220 individuals)
[c] Among participants aged ≥18 years (n = 2549; n = 1131 in known *S. japonicum*-endemic villages)

Living at an elevation ≥2150 m was a significant risk factor for *A. lumbricoides* (OR = 1.51, P <0.001) and *Taenia* spp. (OR = 5.32, P <0.001), but was protective against an infection with *T. trichiura* (OR = 0.31, P = 0.005). *S. japonicum* infections were only found below 2150 m (Table 8.3). Antibodies against cysticerci were associated with lower education, residency at ≥2150 m or in a mountain village, and low socio-economic status (Table 8.4). The same characteristics were protective factors for trichinellosis.

Reported hand washing before meals and after defecation were significantly associated with each other, with higher socio-economic status (quintiles 3-5) and, negatively, with living in a mountain village or in villages situated at an altitude ≥2150 m (all P <0.001, data not shown).

The associations between infection status and hand washing and food consumption are summarized in Table 8.5. Washing hands before meals was protective against *A. lumbricoides* (OR = 0.74, P = 0.011) and hand washing after defecation was a negative predictor for cysticercosis seropositivity (OR = 0.77, P = 0.015). The consumption of raw pork was a positive predictor for *Trichinella* spp. seropositivity (OR = 1.56, P = 0.002).

Table 8.5 Stepwise multiple logistic regression to assess associations between hand washing behaviour, raw food consumption and parasite infection status.

Risk factors	*A. lumbricoides*[a]		*Taenia* spp.[a]		*T. trichiura*[a]		*S. japonicum*[b]		*S. japonicum* ELISA[b]		Cysticercosis ELISA[a]		*Trichinella* spp. ELISA	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Washing hands														
Before meals	0.74 (0.58-0.93)	0.011	0.67 (0.42-1.08)	0.103	0.46 (0.23-0.92)	0.028	*		*		*		1.17 (0.98-1.41)	0.085
After defecation	*		*		*		*		*		0.77 (0.63-0.95)	0.015	*	
Consumption of														
Raw pork	0.51 (0.37-0.69)	<0.001	*		0.34 (0.16-0.73)	0.006	*		*		1.32 (0.93-1.96)	0.119	1.56 (1.18-2.05)	0.002
Raw beef	*		*		2.17 (1.13-4.16)	0.019	0.34 (0.14-0.81)	0.014	0.72 (0.56-0.93)	0.011	*		0.78 (0.64-0.96)	0.019
Raw vegetables	*		*		0.44 (0.14-1.33)	0.145	*		*		*		0.57 (0.36-0.90)	0.015
Raw fish	*		*		*		4.57 (0.95-22.10)	0.059	*		*		*	
Raw water plants	0.79 (0.64-0.98)	0.033	0.54 (0.35-0.83)	0.005	*		*		*		*		0.78 (0.66-0.93)	0.006
Sex:														
Female	**		**		**		**		1.00		1.00		1.00	
Male	**		**		**		**		0.70 (0.56-0.88)	0.003	1.24 (1.03-1.50)	0.023	1.45 (1.24-1.71)	<0.001
Age (years)														
5-9	*		*		**		*		1.00		*		1.00	
10-14	*		*		**		*		2.03 (1.09-3.77)	0.025	*		2.37 (1.57-3.56)	<0.001
15-24	*		*		**		*		3.36 (1.90-5.94)	<0.001	*		3.38 (2.34-4.88)	<0.001
25-39	*		*		**		*		4.86 (2.93-8.06)	<0.001	*		6.25 (4.49-8.69)	<0.001
≥40	*		*		**		*		4.01 (2.43-6.62)	<0.001	*		9.21 (6.60-12.87)	<0.001
Ethnic group														
Bai	**		**		**		1.00		1.00		**		1.00	
Han	**		**		**		4.25 (2.13-8.45)	<0.001	2.36 (1.74-3.22)	<0.001	**		1.99 (1.57-2.52)	<0.001
Livestock breeder	0.56 (0.44-0.70)	<0.001	**		0.45 (0.22-0.90)	0.025	**		*		**		*	
Tobacco grower	*		**		**		*		2.65 (1.68-4.19)	<0.001	1.52 (1.13-2.04)	0.006	*	
Temporary employment	1.71 (0.98-2.98)	0.059	**		**		**		**		**		**	
Resident at altitude ≥2150m	*		4.19 (2.64-6.66)	<0.001	0.32 (0.14-0.74)	0.008	n.a.		n.a.		1.37 (1.04-1.80)	0.027	*	
Socio-economic status														
Most poor	1.00		*		*		**		1.00		1.00		1.00	
Very poor	0.76 (0.56-1.05)	0.093	*		*		**		1.58 (0.95-2.62)	0.078	1.16 (0.83-1.61)	0.392	2.71 (2.06-3.55)	<0.001
Poor	0.55 (0.39-0.77)	<0.001	*		*		**		n. a.		1.56 (1.11-2.19)	0.010	2.05 (1.56-2.69)	<0.001
Less poor	0.51 (0.35-0.73)	<0.001	*		*		**		0.68 (0.48-0.98)	0.037	1.40 (0.94-2.08)	0.099	2.64 (1.97-3.55)	<0.001
Least poor	0.51 (0.34-0.75)	0.001	*		*		**		0.51 (0.36-0.73)	<0.001	1.28 (0.84-1.96)	0.249	2.76 (2.01-3.79)	<0.001
Village location														
Plain area	1.00		*		**		**		**		1.00		1.00	
Mountains	0.76 (0.56-1.03)	0.079	*		**		**		**		1.46 (1.10-1.93)	0.008	0.54 (0.43-0.68)	<0.001

Analyses have been adjusted for demographic indicators, village location and socio-economic status whenever necessary (P <0.1 in bivariate analysis)
OR: odds ratio, CI: confidence interval, P: based on likelihood ratio test, n.a.: not applicable, * Removed at a level of P = 0.15, ** P ≥0.1 in bivariate analysis
[a] All villages (n = 3220 individuals)
[b] In the 13 known *S. japonicum*-endemic villages (n = 1429 individuals)

8.5 Discussion

There is a paucity of community-based studies assessing the prevalence of helminth infections and underlying risk factors in rural China and Southeast Asia more generally (Ohta and Waikagul, 2006). We carried out a study in 35 randomly selected villages in the mountainous county of Eryuan, located in the Yunnan province, which is partially endemic for *S. japonicum*. The analysis of a single stool sample by the Kato-Katz technique resulted in low mean prevalences of soil-transmitted helminths, *S. japonicum* and *Taenia* spp. However, a single blood sample subjected to an ELISA test resulted in high seroprevalences of schistosomiasis japonica, trichinellosis and cysticercosis. Risk factors for infection were determined by bivariate and multiple logistic regression analysis.

Our final study cohort ($n = 3220$) exceeded the estimated minimum sample size by approximately 20%. It included a higher proportion of females and the average age was higher than that of the population initially selected from the community registries. We speculate that the difference arises from out-migration of primarily young males for education and labour. It must also be considered that Chinese villagers usually remain registered in their native village even if they spend most of their time outside the county. Therefore, we assume that our sample is nevertheless representative of the actual resident population.

Possible reasons for the low prevalence of soil-transmitted helminth infections include the climate and recent economic advances, which improved access to clean water, sanitation and anthelmintic drugs (de Silva et al., 2003; Bethony et al., 2006). The prevalence of *A. lumbricoides* was higher among the poor and in those with no or only basic education. The higher prevalence observed in villages located ≥2150 m and in mountain villages probably reflects the generally lower socio-economic status in those areas. A certain fraction of the *A. lumbricoides* infections might also have been acquired elsewhere as the prevalence was especially high in those reporting temporary employment. *T. trichiura* was almost exclusively found at elevations <2150 m. The observed hookworm prevalence was 0.3%. The reason for the low prevalence of hookworm infection could be the cool climate. However, the time delays between stool production by study participants and sample collection by our team and transportation to the laboratory, and loss of hookworm eggs due to time lags of up to several hours between the Kato-Katz thick smear preparation and examination under a microscope could be other reasons (Martin and Beaver, 1968; Dacombe et al., 2007).

Taenia spp. infections and cysticercosis seropositivity were particularly prominent in the poor with no or only basis education, living at altitudes ≥2150 m. Trichinellosis seropositivity,

on the other hand, was more prevalent at lower altitudes, among elderly men, the more affluent and the Han nationality.

The measured seroprevalence rates for schistosomiasis japonica, cysticercosis and especially trichinellosis are high, but we are confident that they accurately represent the local conditions. This assumption is based on the following grounds. Firstly, we used standard tests recommended by the National Institute of Parasitic Diseases in Shanghai. Secondly, the common exposition to risk factors (water contact, low hygiene conditions, raw meat consumption) favours high seroconversion rates. Trichinellosis outbreaks and cysticercosis cases are common in Dali prefecture and Eryuan is the most severely affected county. In the case of the ELISA test for *Trichinella* spp., cross-reactions are known to occur with antibodies against *Toxoplasma* spp., *Echinococcus* spp. and cysticerci. In our study, we found no significant association between cysticercosis and trichinellosis seropositivity ($\chi^{2} = 0.12$, $P = 0.726$).

Tobacco farming was reported by 35.2% of the Han but only 4.3% of the Bai people. Both tobacco growing and being Han were the most prominent risk factors for an infection with *S. japonicum*. Similar results were reported from an endemic area in Sichuan province by Spear et al. (2004) who found that villages devoting a higher percentage of their land to tobacco and vegetable growing had higher prevalences. It is also conceivable that some of the infections were imported. The prevalence of *S. japonicum* in China markedly declined in the 1990s as a result of a concerted effort of the Chinese government, backed by the 10-year World Bank loan project for schistosomiasis control (Chen et al., 2005). Recently, the re-emergence of schistosomiasis japonica was reported in the mountainous areas (Utzinger et al., 2005; Liang et al., 2006). We only found light infections, probably a result of repeated rounds of mass administration of praziquantel.

The achievements of the Chinese schistosomiasis control programme bring along new challenges. For example, the low infection intensity makes the Kato-Katz technique increasingly unreliable and the examination of a single stool sample can result in a considerable underestimation of the true prevalence (Yu et al., 1998; Zhu, 2005; Wang et al., 2006a). The increased sampling and diagnostic effort in three villages resulted in the detection of 21 additional *S. japonicum* infections. Interestingly, in one of these three villages, the Kato-Katz method performed on a single stool specimen failed to identify any of the four cases, and in the other two villages, the more intensive diagnostic approach detected additional cases. With the advent of sensitive serological tests for schistosomiasis japonica in the 1980s, it has become a common practice in China to first screen the population by ELISA, followed by

stool examination using the Kato-Katz technique among seropositives. In our study, this would have left undetected 3 of the 38 cases in the "known" schistosome-endemic villages (sensitivity: 92.1%). An additional 4 egg-positive cases were detected in villages previously thought to be *S. japonicum*-free. The standard tools for schistosomiasis control in China were developed in, and adapted to, the larger and ecologically more uniform endemic areas in eastern China. However, the focalized transmission in mountainous areas and the resulting small-scale classification into *Oncomelania*-infested, hence endemic communities, and non-endemic villages can result in residents of non-endemic villages being infected but not covered by control campaigns.

Higher education and socio-economic status were positive predictors for hand washing, which in turn was protective against *A. lumbricoides*, *T. trichiura* and cysticercosis seropositivity. Whilst 13.5% of the least poor families lacked sanitation facilities, the respective percentage in the 'most poor' families was 86.3%. The higher prevalence of *Taenia* spp. infection and cysticerci-specific antibodies in poorer population strata and in mountainous areas could result from the joint effects of more precarious hygiene conditions, a higher prevalence of small-holder pig farming than in more affluent areas (94.9% *versus* 62.3%), and the absence of praziquantel-based mass chemotherapy in non-schistosome-endemic areas. Praziquantel is the drug of choice for the treatment of taeniasis and cysticercosis (Ito et al., 2003; Chen et al., 2004). Raw pork or raw beef was consumed by 90.0% and 25.9% of the participants, respectively. Whilst the consumption of raw pork was equally common in Han and Bai and across all socio-economic strata, Han consumed raw beef more frequently (49.4%) than Bai (20.1%). The prevalence of raw beef consumption steadily increased with socio-economic status, from 5.9% among the poorest to 45.3% among the richest. The almost universal consumption of raw pork prevents its conclusive association with any infection. Therefore, the observed association with trichinellosis might be a chance finding, but is supported by observations in other areas of China (Wang et al., 2006b).

We observed an inverse relationship between the mean socio-economic status of the families and distance from the main road. The highest proportions of very poor and most poor families were found in mountainous areas without road access. In our future work, we plan to carry out more detailed analyses of the relationship between the disparity in socio-economic conditions and the local epidemiology of parasitic diseases taking into account between and within village variation. This kind of information will clarify the observed relations and aid in the tailoring of setting-specific control approaches.

Recently, soil-transmitted helminth infections and other so-called neglected tropical diseases have attracted new attention (Molyneux et al., 2005; Hotez et al., 2006; Utzinger and de Savigny, 2006). It has also been argued that integration of parasite-specific control programmes could use synergies and hence become more cost-effective (Brady et al., 2006; Engels and Savioli, 2006; Fenwick, 2006). Research, monitoring and control of schistosomiasis have a long history in China (Utzinger et al., 2005) but until now, the respective infrastructure has hardly been used for other public-health activities. The present study was carried out using staff and tools available in local schistosomiasis control institutions. As the prevalence of *S. japonicum* declines, there is scope to use this established control and surveillance infrastructure for other parasites, especially soil-transmitted and food-borne helminths.

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9. Spatial risk profiling of *Schistosoma japonicum* in Eryuan county, Yunnan province, China

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9.1 Abstract

Bayesian spatial risk profiling holds promise to enhance our understanding of the epidemiology of parasitic diseases, and to target interventions in a cost-effective manner. Here, we present findings from a study using Bayesian variogram models to map and predict the seroprevalence of *Schistosoma japonicum* in Eryuan county, Yunnan province, China, including risk factor analysis. Questionnaire and serological data were obtained through a cross-sectional survey carried out in 35 randomly selected villages with 3220 people enrolled. Remotely-sensed environmental data were derived from publicly available databases. Bivariate and non-spatial Bayesian multiple logistic regression models were used to identify associations between the local seroprevalence and demographic (i.e. age and sex), environmental (i.e. location of village, altitude, slope, land surface temperature and normalized difference vegetation index) and socio-economic factors. In the spatially-explicit Bayesian model, *S. japonicum* seroprevalence was significantly associated with sex, age and the location of the village. Males, those aged below 10 years and inhabitants of villages situated on steep slopes (inclination ≥20°) or on less precipitous slopes of >5° above 2150 m were at lower risk of seroconversion than their respective counterparts. Our final prediction model revealed an elevated risk for seroconversion in the plains of the eastern parts of Eryuan county. In conclusion, the prediction map can be utilized for spatial targeting of schistosomiasis control interventions in Eryuan county. Moreover, *S. japonicum* seroprevalence studies might offer a convenient means to assess the infection pressure experienced by local communities, and to improve risk profiling in areas where the prevalence and infection intensities have come down following repeated rounds of praziquantel administration.

Keywords: *Schistosoma japonicum*, seroprevalence, risk mapping and prediction, Bayesian variogram models, geographical information system, remote sensing, China.

9.2 Introduction

Schistosoma japonicum is a blood-dwelling fluke that is plaguing humans and over 40 domestic and wild animal species in China, Indonesia and the Philippines. Adult female *S. japonicum* flukes produce eggs, about half of which are trapped in host tissues where they elicit inflammatory immune reactions and organ damage. The remaining eggs are released to the environment via the faeces. Miracidia hatch upon contact of the eggs with freshwater and infect amphibious intermediate host snails of the genus *Oncomelania*. The emerging cercariae close the transmission cycle by percutaneously penetrating mammalian end hosts during water contact (Ross et al., 2001; Gryseels et al., 2006). In China, it is currently estimated that between 700,000 and 850,000 individuals are infected with *S. japonicum*, among the 40 million people at risk of contracting the disease (Zhou et al., 2005). Since the mid-1950s, the Chinese government is implementing a multitude of control measures, including environmental management targeted against the intermediate host snail for transmission containment and mass drug administration for morbidity control (Utzinger et al., 2005). Achievements made to date hold promise for ultimately attaining the final aim of transmission interruption and elimination of schistosomiasis from China (Zhou et al., 2005).

Remote sensing (RS) and geographical information system (GIS) techniques are increasingly used in public health and veterinary sciences (Hay, 2000; Rinaldi et al., 2006), and have given new impetus to the study of infectious diseases (Rogers and Randolph, 2003; Brooker et al., 2006). Their capacity to acquire and process large amounts of spatially-explicit data offers new prospects for the assessment of environmental factors that influence the epidemiology of neglected tropical diseases, including schistosomiasis (Bergquist, 2001; Brooker, 2002; Brooker and Utzinger, 2007). In China, GIS and RS have been widely and effectively utilized for the study and control of *S. japonicum* (Yang et al., 2005c). Yet, the application of GIS and RS has primarily focussed on the mapping of human infections and *Oncomelania hupensis* habitats at different scales, the prediction of suitable snail habitats in eastern China, and the assessment of the potential impact of environmental transformations and climate change (Yang et al., 2005c).

Recently, the integration of a diversity of datasets for risk profiling of malaria and neglected tropical diseases has been advanced by the development of spatial statistical methods, e.g. Bayesian methods and Markov chain Monte Carlo (MCMC) inference (Basanez et al., 2004; Gemperli et al., 2004). Subsequently, application of these techniques have been successfully extended for risk profiling of schistosomiasis in different African settings (Raso

et al., 2005a; Clements et al., 2006). Studies in the mountainous region of Man in western Côte d'Ivoire, for example, suggested that the risk of infection with *S. mansoni* at non-sampled locations could be predicted by demographic, ecological and socio-economic data obtained from questionnaires and satellite-derived environmental data (Raso et al., 2005a; Beck-Wörner et al., 2007).

The objectives of this study were: (i) to develop a spatially-explicit statistical model by integrating epidemiological and remotely-sensed environmental data for risk profiling of *S. japonicum* seroconversion and infection in a mountainous area in China; and (ii) to explore the potential of serological data for the appreciation of the infection pressure in this setting, where praziquantel has been repeatedly administered over the past several years.

9.3 Materials and methods

9.3.1 Study area

Our study was carried out in Eryuan, a mountainous county in the northwest of Yunnan province, China. Eryuan county covers an area of approximately 3000 km^2, stretching from 25.80° to 26.43° N latitude and from 99.54° to 100.43° E longitude. Rains mainly occur during the summer monsoon period (June-September). The elevation of the mountainous and partially forested western part of the county is between 1700 m and 3000 m above sea level. The eastern part features fertile, mountain-framed plains at altitudes of 1950-2150 m. The autochthonous Bai and the immigrated Han ethnic groups are both engaged in agriculture and animal husbandry. The latter primarily live in the eastern plains. Whilst intensive irrigated farming dominates in plain areas and on terraced mountain slopes, rain-fed agriculture and livestock breeding dominate elsewhere. Infrastructure development started in the plains and is progressing into the mountainous areas.

9.3.2 Cross-sectional parasitological and questionnaire surveys

The epidemiological data were derived from a cross-sectional community-based survey described in detail elsewhere (Steinmann et al., 2007). In brief, the study included 35 villages that were identified by randomly selecting cells of a 3.5 x 3.5 km-grid laid over a map of Eryuan county. In every village, 35 families were randomly selected from available community registries. Families were visited in November and December 2005 and the purpose and procedures of the study were explained. In case no adult family member was

around during our first encounter, a neighbouring family was invited to participate. All family members aged ≥5 years were enrolled.

The following field procedures were employed. Firstly, individual and family-level risk factors were obtained by means of pre-tested questionnaires. The individual questionnaire recorded demographic features and health-related behaviour. Study participants <15 years of age were usually assisted by their parents/legal guardians in answering the questions. The household questionnaire was addressed to the head of the family and assessed general living conditions, and ownership of household assets, livestock and farmland. Secondly, a venous blood sample was collected from each participant, centrifuged on the spot and forwarded to the research station where it was frozen, pending analysis. Thirdly, labelled plastic bags for the collection of stool specimens were handed out to all participants. One stool sample was obtained per individual and forwarded to the schistosomiasis control station at the day of collection.

Finally, the geographical coordinates of participating households were recorded, using a hand-held global positioning system (GPS) receiver (Garmin Ltd.; Olathe, USA). The sustained schistosomiasis control and surveillance activities in China have resulted in the classification of all villages into different endemicity levels, taking into account historical and contemporary results from parasitological surveys. Hence, the study villages were classified into schistosome-endemic and non-endemic settlements on the basis of this prior knowledge.

In the laboratory, stool samples were first screened for helminth eggs by reading four 42 mg Kato-Katz thick smears per sample under a light microscope (Katz et al., 1972). Eggs were counted and recorded separately for each helminth species. For quality control, 5% of the slides were re-examined by the senior laboratory technician. A commercially-available enzyme-linked immunosorbent assay (ELISA) was used to test the serum samples for IgG antibodies against *S. japonicum* (Shenzhen Combined Biotech Co. Ltd.; Shenzhen, China). This ELISA test exhibits high sensitivity and specificity and has been extensively used in the national schistosomiasis control programme of China (Zhu, 2005). The standardized positive and negative control sera included in the test kit were employed and the cut-off values specified in the manual were applied for the discrimination between seropositive and seronegative individuals. Cysticerci and *Trichinella* spp. were also tested for by ELISA (Hai Tai Co. Ltd.; Zhuhai, China) according to the manufacturer's instructions.

9.3.3 Demographic and socio-economic data

The demographic data (i.e. age and sex) were derived from the individual questionnaire. The socio-economic data were extracted from the household questionnaire. We utilized an asset-based approach to stratify the participating families into wealth quintiles. This approach, initially developed in India (Filmer and Pritchett, 2001), and then successfully applied in rural and urban areas of East and West Africa (Armstrong Schellenberg et al., 2003; Raso et al., 2005b; Matthys et al., 2006), was further adapted to the current setting in rural China.

9.3.4 Environmental data

Eight-day composite night-time land surface temperature (LST) and 16-day composite normal difference vegetation index (NDVI) data at a nominal resolution of 1 x 1 km were obtained for the periods 1 December 2004 to 28 February 2005 and from 1 March 2005 to 31 May 2005, respectively. These data were obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS), made available through the Land Processes Distributed Active Archive Center, U.S. Geological Survey (USGS) (http://edcdaac.usgs.gov/dataproducts.asp). Individual scenes were mosaicked, if necessary, and the required bands reprojected utilizing the appropriate functions of the reprojection tool of MODIS (version 3.3). Scenes were resized in ENVI 4.0 (Research Systems, Inc., Boulder, USA) and values extracted for village centroids and all other pixels. Subsequently, median values over the considered periods were computed. A 90 m high-resolution Shuttle Radar Topography Mission (SRTM)-derived digital elevation model (DEM) was downloaded from the USGS EROS data center (http://eros.usgs.gov/). Slopes (in degrees [°]) were calculated for pixels with similar spatial extent than the LST and NDVI data, using ArcMap version 9.1 (Environmental Systems Research Institute Inc., Redlands, USA) and elevation and slope data were extracted as outlined above. It is known that the intermediate host snail of *S. japonicum* mainly lives in irrigation canals in Eryuan county. In addition, preceding analyses indicated that most *S. japonicum* infections occurred at an elevation below 2150 m (Steinmann et al., 2007). Therefore, an attempt was made to classify the environment into plain regions (slope <5°) and sloped areas below or above 2150 m. The underlying assumption was that irrigation is more common in plain areas when compared to mountain slopes. However, there are terraced areas in Eryuan county but usually not on steep mountain slopes. For visualizing purposes, all spatial data were displayed in ArcMap version 9.1.

9.3.5 Data management and statistical analysis

EpiData version 3.1 (EpiData Association; Odense, Denmark) was used for double entry and validation of the questionnaire data. Statistical analysis, including bivariate and multiple logistic regression analyses, were performed in STATA version 9.2 (StataCorp LP; College Station, USA). Potential risk factors for seropositivity were subjected to bivariate logistic regression analysis done for all villages, and for schistosome-endemic and non-endemic villages separately. Demographic, socio-economic and environmental risk factors which proved to be significant for the full dataset, but were not obviously correlated with each other, were included in the Bayesian non-spatial and spatial multiple logistic regression models.

The infection status Z_{ijk} of individual k within household j at village i was assumed to follow a Bernoulli distribution ($Z_{ijk} \sim Ber(p_{ijk})$), whereas p_{ijk} is a measure for the risk of seropositivity for individual k within household j in village i. Multiple logistic regression models were fitted with exchangeable random effects ($u_j, j = 1,2,3,...J$) at the household level and spatially correlated random effects (φ i, $I = 1,2,3,...N$) at the village level. Covariates and random effects were modelled on the log*it* scale, that is log*it*(p_{ijk}) = $X_{ijk}\beta^{T} + u_j + \varphi_2$. It is assumed that $u_j \sim N(0,\sigma_1^2), j = 1,...J$ and $\varphi \sim MVN(0,\Sigma)$, where σ_1^2 is the between-household variation. Σij = $\sigma_2^{\ 2}$ and R(d_{ij}, ρ) = exp(- $\rho\, d_{ij}$), $\sigma_2^{\ 2}$ is the spatial variation, d_{ij} the shortest straight-line distance between village i_1 and i_2, and ρ is the rate of how correlation decreases with distance. We assumed an exponential spatial correlation, i.e. exp($-\rho d_{ij}$). The minimum distance in meters (m) at which spatial correlation between village locations drops below 5%, is known as the range of geographical dependency, which is expressed by $\delta = 3/\rho$.

We chose Normal prior distributions with a mean equal to 0 and a variance equal to 100 for the regression coefficients, inverse gamma prior distributions for ρ, $\sigma_1^{\ 2}$ and $\sigma_2^{\ 2}$, and gamma prior distribution for ρ. A sensitivity analysis for ρ considering informative and non-informative prior distributions has been undertaken. The best fitting model, based on the smallest Bayesian deviance information criterion (DIC) used as a goodness of fit measure, with an informative gamma distribution (mean equal to 1 and variance equal to 1) for ρ, has been selected and used for spatial prediction (Spiegelhalter et al., 2002). The model with the smallest DIC was considered as the best fitting one and results will be presented for this model. The model parameters were estimated employing MCMC simulation (Gelfand and

Smith, 1990). A single chain sampler with a burn-in of 10,000 iterations was run. The ergodic averages of the model parameters were examined for convergence.

9.3.6 Ethical considerations and anthelmintic treatment

The institutional review boards of the Swiss Tropical Institute (Basel, Switzerland) and the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, China) granted approval for this study. The local authorities were informed about the objectives and procedures of the investigation. Written informed consent was obtained from the heads of the participating families.

At the end of the study, a single 40 mg/kg oral dose of praziquantel was given to individuals with a *S. japonicum* infection, and a single 400 mg oral dose of albendazole was administered to those with a soil-transmitted helminth infection. *Taenia* spp. infections were treated with a single dose of 600 mg praziquantel for adults and 400 mg for children. All drugs were administered free of charge.

9.4 Results

9.4.1 Study cohort

Complete questionnaire, parasitological and serological data were obtained from 3220 individuals from 1154 families, which formed our final study cohort. There were 1793 females (55.7%) and the age ranged between 5 and 81 years. The age structure was as follows: 5-9 years (8.6%), 10-14 years (7.7%), 15-24 years (12.9%), 25-39 years (35.0%) and ≥40 years (35.8%). Four-fifth of the participants belonged to the Bai minority, and the remaining 20% were Han Chinese. The large majority of the study population were farmers (80%), whereas 16% were students. Animal ownership was almost universal, particularly pigs (reported by 78% of the families).

With regard to the socio-economic status of the participating families, our household-based asset approach revealed significant spatial heterogeneities in the composition of the village population. The proportion of low socio-economic classes in the villages increased with the distance from the eastern plain, with 99.7% of the least poor families living at an elevation below 2150 m.

9.4.2 Parasitological findings

Parasitological data and risk factors for infection and seroconversion, as assessed by bivariate and multiple logistic regression analyses, have been presented elsewhere (Steinmann et al., 2007). In brief, eggs of five different helminth species were observed, namely *Ascaris lumbricoides* (15.4%), *Taenia* spp. (3.5%), *Trichuris trichiura* (1.7%), *S. japonicum* (1.3%) and hookworms (0.3%). In the 13 known schistosome-endemic villages, the prevalence of *S. japonicum* was 2.7%. Interestingly, four *S. japonicum* infections were discovered in villages that were considered as non-endemic. The seroprevalence of *Trichinella* spp. and cysticerci-specific antibodies was 58.8% and 18.5%, respectively. Prominent risk factors for a *S. japonicum* infection in endemic villages included ethnicity (i.e. Han Chinese), growing tobacco and residency in plain areas and at an elevation <2150 m.

9.4.3 Risk factors for S. japonicum *seropositivity*

The location and schistosomiasis seroprevalence of each village is displayed in Figure 9.1 and the key findings regarding the *S. japonicum* seropositives are summarized in Table 9.1. The mean seroprevalence of *S. japonicum* in the entire study area was 27.1%. It varied between 16.9% and 84.8% (mean: 49.5%) in known schistosome-endemic and between 1.2% and 32.1% (mean: 9.2%) in non-endemic villages. Risk factors for seroconversion among residents of non-endemic villages included age (≥25 years), socio-economic status (less poor: odds ratio (OR) = 1.95, 95% confidence interval (CI) = 1.17-3.25; least poor: OR = 2.10, 95% CI = 1.21-3.65) and lease of irrigated land with a surface exceeding 1.5 mǔ (1 mǔ = 666.7 m^2). Residency at an altitude ≥2150 m (OR = 0.55, 95% CI = 0.40-0.76) and living on slopes >5° was protective (altitudes <2150 m: OR = 0.59, 95% CI = 0.36-0.95; altitude ≥2150 m: OR = 0.38, 95% CI = 0.25-0.60). A higher socio-economic status and residency in a plain area were strong risk factors for seroconversion in the entire study population but the former was protective in known schistosome-endemic areas. The median night-time LST between December 2004 and February 2005 was a predictor for seropositivity in Eryuan county, whereas the median NDVI between March and May 2005 was negatively correlated with seropositivity.

9.4.4 Risk mapping and prediction

The non-spatial multiple logistic regression model for all surveyed villages included significant demographic, socio-economic and environmental covariates identified in the

bivariate logistic regression analysis. Key findings derived from the models are summarised in Table 9.2. In the bivariate analysis, there were significant associations between the serostatus and age, sex, socio-economic status, village location, the median night-time LST between December 2004 and February 2005 and the median NDVI between March and May 2005. Females and individuals aged ≥10 years, compared to the youngest age group (5-10 years), were at a higher risk of seroconversion. On the other hand, a higher socio-economic status was a protective factor. Tobacco growing and working irrigated farmland was a risk factor for seropositivity. Inhabitants of villages located on slopes with an inclination >5° and at an altitude above 2150 m, as well as those in villages on mountainsides with an inclination >10° at any elevation, were less likely to be seropositive. A higher median NDVI between March and May 2005 at the location of the study villages was protective against seroconversion.

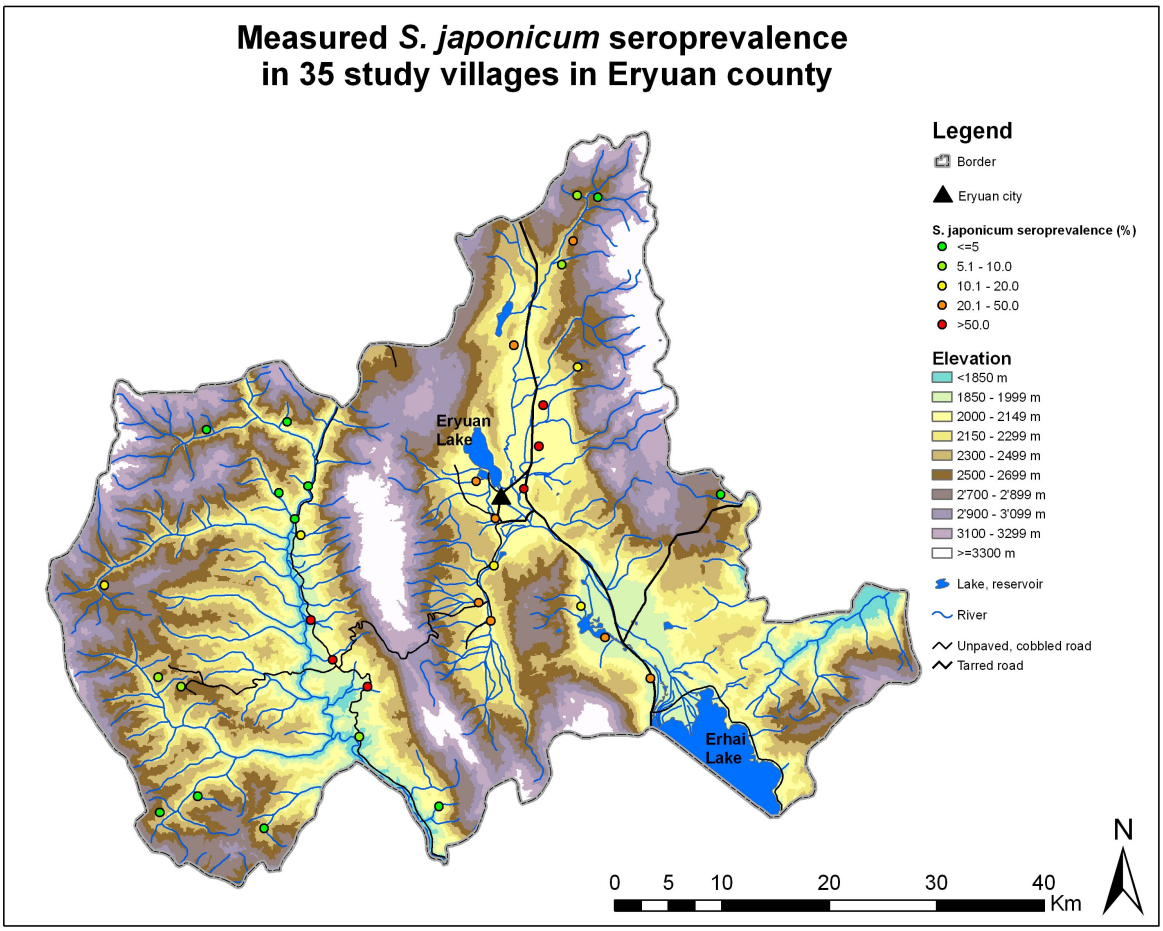

Figure 9.1 Measured *S. japonicum* seroprevalence in 35 study villages in Eryuan county, Yunnan province, China. Also depicted on the map are the elevation, water bodies and roads.

The results of the spatially-explicit logistic regression model, which included the same covariates as those described before, are also shown in Table 9.2. Whilst sex and age remained significant risk factors for seroconversion, the other covariates showed no statistical significance, with the exception of the village location. In fact, inhabitants of villages situated on steep slopes (inclination ≥20°) or on a less precipitous slope of >5° above 2150 m were at a lower risk of seropositivity than people living in plain areas.

Table 9.1 Bivariate logistic regression analyses of the relationship between the *S. japonicum* serostatus and demographic indicators, socio-economic status of the families, village location and environmental covariates in Eryuan county, Yunnan province, China, stratified by known endemicity of *S. japonicum* at the village level.

	S. japonicum seropositivity (based on ELISA results)					
	All villages		Schistosome-endemic villages		Non-endemic villages	
Prevalence (%) at village level (range)	27.1 (1.2-84.8)		49.5 (16.9-84.8)		9.2 (1.2-32.1)	
Risk factors	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sex						
Female	1.00		1.00		1.00	
Male	0.79 (0.67-0.92)	0.003	0.72 (0.58-0.89)	0.002	0.76 (0.55-1.06)	0.108
Age (years)						
5-9	1.00		1.00		1.00	
10-14	2.06 (1.27-3.34)	0.004	2.39 (1.35-4.22)	0.003	1.46 (0.38-5.56)	0.576
15-24	2.27 (1.46-3.52)	<0.001	3.23 (1.91-5.48)	<0.001	2.36 (0.77-7.24)	0.134
25-39	3.68 (2.48-5.46)	<0.001	4.80 (3.02-7.64)	<0.001	4.79 (1.72-13.31)	0.003
≥40	3.29 (2.22-4.88)	<0.001	3.48 (2.20-5.52)	<0.001	4.84 (1.74-13.47)	0.003
Education if age ≥18 years						
Illiterate	1.00		1.00		1.00	
≤Junior middle school	2.06 (1.69-2.51)	<0.001	1.10 (0.81-1.49)	0.533	1.31 (0.92-1.87)	0.141
≥High middle school	1.55 (0.99-2.42)	0.053	0.65 (0.37-1.15)	0.137	1.16 (0.44-3.04)	0.768
Ethnic group						
Bai	1.00		1.00		1.00	
Han	2.37 (1.97-2.84)	<0.001	2.40 (1.87-3.07)	<0.001	1.13 (0.73-1.75)	0.576
Tobacco grower	2.03 (1.06-2.57)	<0.001	4.32 (2.91-6.41)	<0.001	0.59 (0.30-1.14)	0.115
Livestock breeder	1.97 (1.68-2.31)	<0.001	1.20 (0.97-1.48)	0.090	1.08 (0.76-1.53)	0.665
Temporary employment	0.97 (0.59-1.60)	0.920	1.44 (0.63-3.26)	0.383	1.57 (0.73-3.36)	0.250

	S. japonicum seropositivity (based on ELISA results)					
	All villages		Schistosome-endemic villages		Non-endemic villages	
Prevalence (%) at village level (range)	27.1 (1.2-84.8)		49.5 (16.9-84.8)		9.2 (1.2-32.1)	
Risk factors	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Socio-economic status						
Most poor	1.00		1.00		1.00	
Very poor	3.47 (2.35-5.12)	<0.001	n.a.		1.51 (0.96-2.36)	0.075
Poor	3.95 (2.69-5.79)	<0.001	0.71 (0.44-1.14)	0.158	0.98 (0.59-1.63)	0.949
Less poor	7.77 (5.38-11.22)	<0.001	0.54 (0.35-0.82)	0.004	1.95 (1.17-3.25)	0.011
Least poor	8.41 (5.85-12.10)	<0.001	0.48 (0.32-0.73)	0.001	2.10 (1.21-3.65)	0.008
Tenancy of irrigated agricultural land						
No irrigated land	1.00		1.00		1.00	
0.1-1.5 mǔ[a]	3.03 (2.19-4.20)	<0.001	0.78 (0.53-1.13)	0.188	1.37 (0.90-2.10)	0.141
1.6-2.5 mǔ[a]	7.63 (5.67-10.28)	<0.001	n.a.		2.01 (1.21-3.35)	0.007
2.6-3.5 mǔ[a]	8.94 (6.67-12.02)	<0.001	0.98 (0.74-1.30)	0.905	3.15 (1.92-5.18)	<0.001
>3.5 mǔ[a]	16.62 (12.34-22.39)	<0.001	1.61 (1.22-2.12)	0.001	3.43 (1.59-7.41)	0.002
Resident at altitude ≥2150m	0.13 (0.10-0.16)	<0.001	n.a.		0.55 (0.40-0.76)	<0.001
Slope						
<10°	1.00		1.00		1.00	
10 to <20°	0.16 (0.13-0.20)	<0.001	0.40 (0.27-0.57)	<0.001	0.49 (0.35-0.69)	<0.001
≥20°	0.10 (0.07-0.14)	<0.001	0.16 (0.11-0.23)	<0.001	0.13 (0.07-0.26)	0.001
Village location						
Plain area (slope ≤5°)	1.00		1.00		1.00	
Slope >5°, altitude <2150m	0.47 (0.39-0.56)	<0.001	0.57 (0.46-0.70)	<0.001	0.59 (0.36-0.95)	0.032
Slope >5°, altitude ≥2150m	0.08 (0.06-0.11)	<0.001	n.a.		0.38 (0.25-0.60)	<0.001
Median night-time LST[b]	1.47 (1.39-1.56)	<0.001	1.03 (0.94-1.13)	0.561	1.10 (0.99-1.23)	0.067
Median NDVI[c]	0.028 (0.02-0.005)	<0.001	1.88 (0.80-4.44)	0.149	3.22 (0.68-15.20)	0.140

OR: odds ratio; CI: confidence interval, n.a.: not assessed
[a] 1 mǔ = 666.7 m^2
[b] LST: land surface temperature; period: December 2004 to February 2005
[c] NDVI: normalized difference vegetation index; period: March to May 2005

Table 9.2 Non-spatial and spatially-explicit multiple logistic regression model for the analysis of the relationship between the *S. japonicum* serostatus and demographic indicators, socio-economic status of the families, village location and environmental covariates in the 35 study villages in Eryuan county, Yunnan province, China.

Risk factor	*S. japonicum* seropositivity (based on ELISA results)			
	Non-spatial model		Spatial model	
	OR	95% CI	OR	95% BCI
Sex				
Female	1.00		1.00	
Male	0.75	0.62-0.90	0.73	0.59-0.88
Age (years)				
5-9	1.00		1.00	
10-14	2.45	1.40-4.03	2.56	1.37-4.42
15-24	3.05	1.83-4.85	4.48	2.54-7.50
25-39	4.69	2.99-7.15	7.11	4.3-11.39
≥40	3.70	2.37-5.62	5.70	3.45-9.11
Ethnic group				
Bai	1.00		1.00	
Han	0.82	0.62-1.07	0.82	0.50-1.25
Tobacco grower	1.91	1.34-2.65	1.31	0.82-1.97
Socio-economic status				
Most poor	1.00		1.00	
Very poor	0.99	0.57-1.59	1.37	0.73-2.34
Poor	0.69	0.39-1.12	1.04	0.52-1.86
Less poor	0.76	0.42-1.26	1.10	0.54-2.00
Least poor	0.59	0.32-0.99	0.79	0.38-1.46
Tenancy of irrigated agricultural land				
No irrigated land	1.00		1.00	
0.1-1.5 mǔ[a]	0.97	0.54-1.61	0.80	0.40-1.42
1.6-2.5 mǔ[a]	1.56	0.85-2.63	0.96	0.47-1.75
2.6-3.5 mǔ[a]	1.83	1.00-3.08	0.93	0.46-1.70
>3.5 mǔ[a]	3.02	1.65-5.13	1.09	0.52-2.03
Slope				
<10°	1.00		1.00	
10 - <20°	0.46	0.34-0.61	0.58	0.11-1.76
≥20°	0.19	0.13-0.28	0.25	0.04-0.81
Village location				
Plain area (slope ≤5°)	1.00		1.00	
Slope >5°, altitude <2150m	0.91	0.69-1.17	0.83	0.19-2.26
Slope >5°, altitude ≥2150m	0.25	0.13-0.43	0.19	0.02-0.69
Median night-time LST[b]	0.98	0.93-1.02	0.97	0.88-1.04
Median NDVI[c]	0.08	0.03-0.16	0.25	<0.01-1.52
Sigma (spatial variation)			1.36	1.27-2.56
Sigma1 (non-spatial variation)			0.07	0.06-0.15
Phi (smoothing parameter)			0.00123	0.00109-0.00276
Deviance information criterion (total)	2967.0		2698.9	

Odds ratios (OR) are shown with 95% confidence interval (CI) and 95% Bayesian credible interval (BCI)
[a] 1 mǔ = 666.7 m^2
[b] LST: land surface temperature; period: December 2004 to February 2005
[c] NDVI: normalized difference vegetation index; period: March to May 2005

The predicted schistosomiasis seroprevalence at non-sampled locations in Eryuan county is displayed in Figure 9.2. Figure 9.3 shows the corresponding standard error of the prediction. The highest prevalences are predicted for the plain areas between Erhai Lake and Eryuan Lake, and for the wide valleys in the western part of the county wherever the mountain slopes are not too steep and the elevation is lower than in the eastern plains. Spatial correlation between two locations dropped below 5% at a distance of 2.5 km.

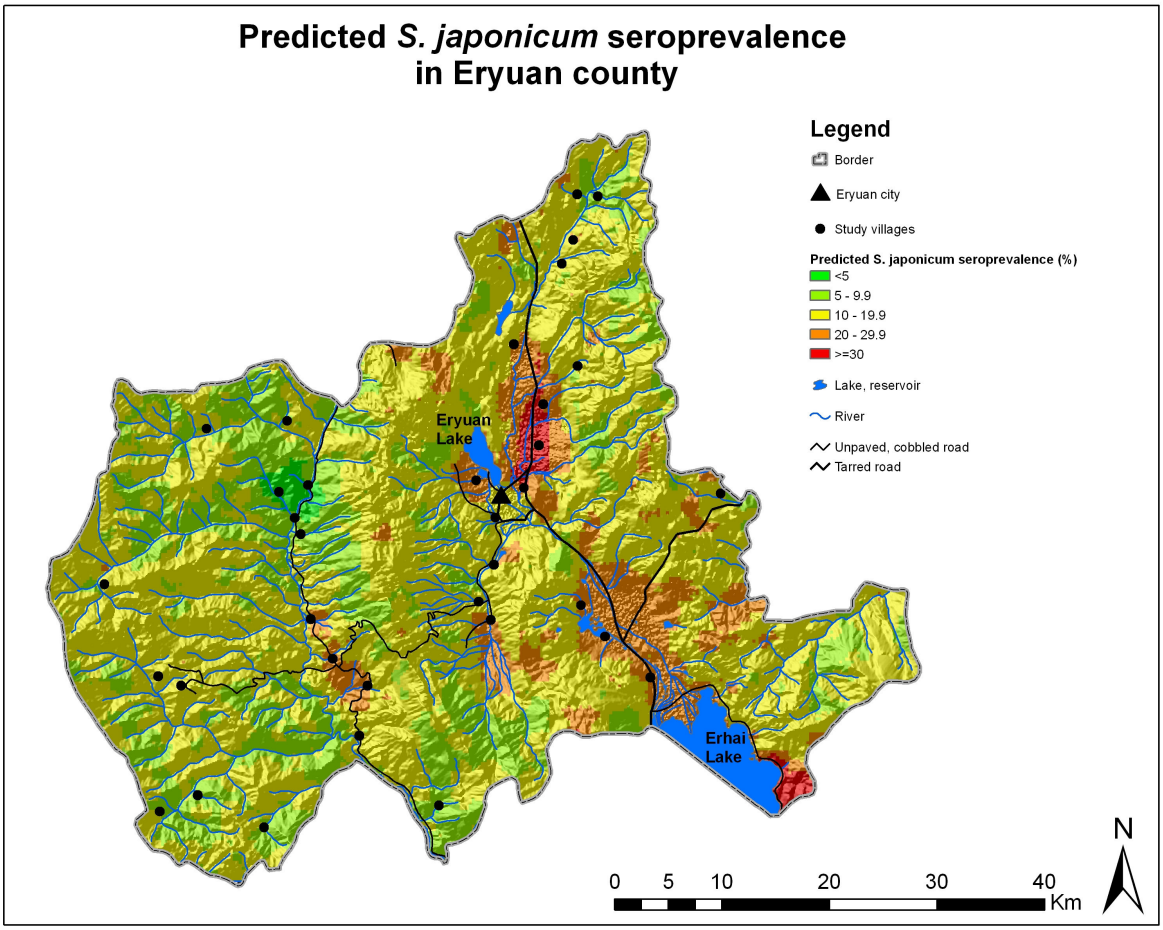

Figure 9.2 Map of the predicted *S. japonicum* seroprevalence in Eryuan county, Yunnan province, China. The prediction is based on the spatially-explicit logistic regression model and the smoothed map was produced by an exponential kriging approach.

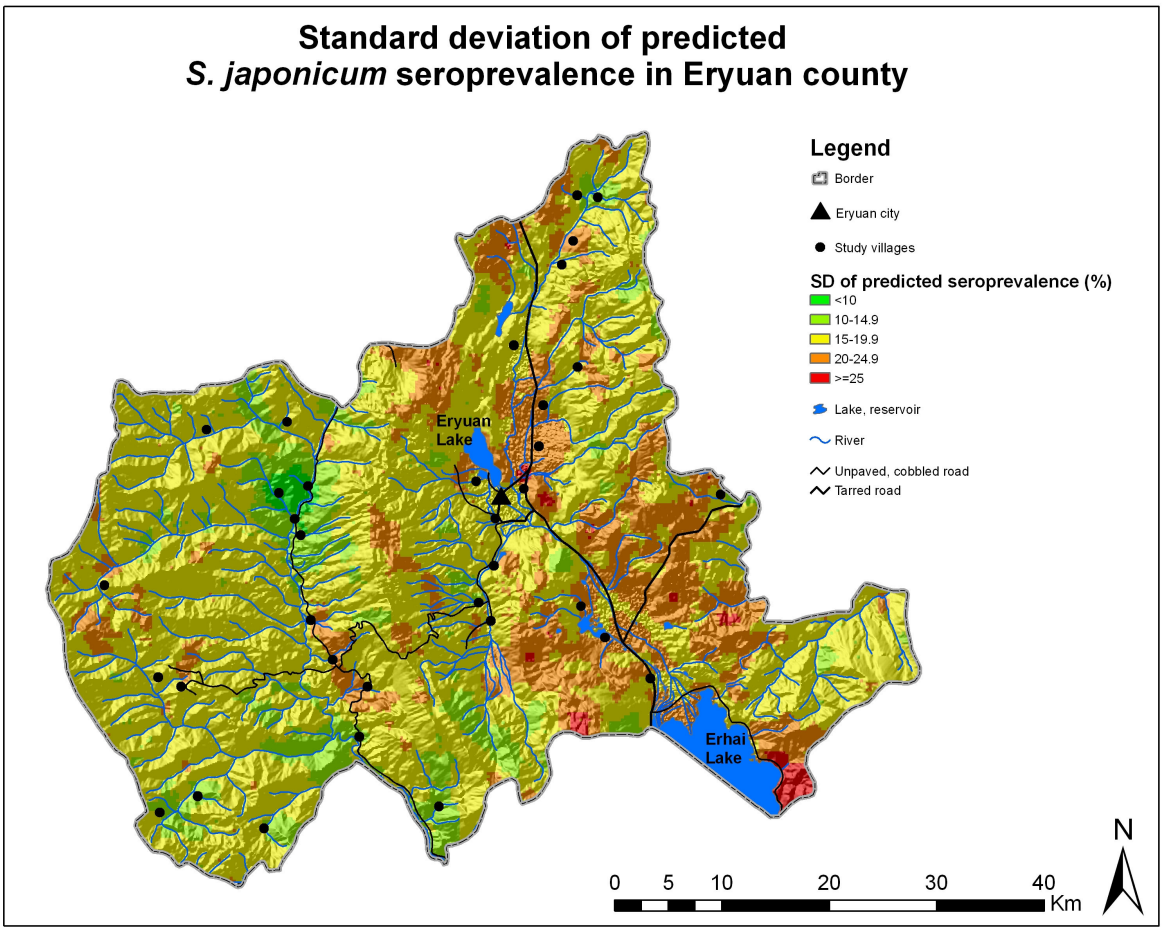

Figure 9.3 Map of the standard error of the predicted *S. japonicum* seroprevalence in Eryuan county, Yunnan province, China. The prediction is based on the spatially-explicit logistic regression model and the smoothed map was produced by an exponential kriging approach.

9.5 Discussion

To our knowledge, this is the first attempt to predict the seroprevalence of *S. japonicum* in a mountainous part of Yunnan province in southwest China. We employed a widely used diagnostic assay, i.e. ELISA (Wu, 2002; Zhu, 2005), and screened 3220 individuals, aged 5-81 years, randomly selected from 35 villages in Eryuan county. Demographic and socio-economic data, obtained by questionnaires, and remotely-sensed environmental data were utilized for risk factor analysis for seroconversion, and to map and predict the seroprevalence at sampled and non-sampled locations in the study area.

Both the bivariate analysis and the non-spatial model identified various demographic, socio-economic and environmental risk factors for seroconversion. In the spatial model, however, the only risk factors that remained significant were sex, age, and two covariates describing the village location with regard to slope and altitude. Age, sex and altitude were

also identified as risk factors for an infection with *S. mansoni* in a previous study focussing on schoolchildren living in a mountainous area of Côte d'Ivoire (Raso et al., 2005a). In addition, that study reported a strong correlation between infection and the socio-economic status, whereas we did not find such a relationship. The Côte d'Ivoire study found that the environmental covariates, i.e. LST and NDVI, were less important determinants of the local prevalence if spatial correlation was taken into account. On county level and higher administrative units, however, these and other environmental covariates have been identified as important predictors for schistosomiasis japonica (Yang et al., 2005a; Yang et al., 2005c). The development of a DEM and further spatial analyses of the Côte d'Ivoire data found that proximity to streams with an order 3 (according to a classification proposed by Strahler in the mid-1950s) was a good predictor for a *S. mansoni* infection, most likely explained by the distribution of *Biomphalaria pfeifferi*, the intermediate host snail of intestinal schistosomiasis in Côte d'Ivoire (Beck-Wörner et al., 2007).

In Eryuan county, *O. hupensis* is mainly found along irrigation and drainage canals and ponds. Hence, we speculate that humans are predominantly infected during occupational activities in irrigated areas (Huang and Manderson, 2005). The higher seroprevalence among older age groups supports this notion. Indeed, we found that tobacco farmers and those working in large irrigated areas are at higher risk of seropositivity. Irrigated agriculture is mainly performed in the plains or on gently inclined slopes. People living in these areas were at higher risk of seroconversion than those residing on steeper mountain slopes. Interestingly, the plain areas are economically more advanced, and most of the families with higher socio-economic status live in the plains (Steinmann et al., 2007). This observation could, at least partly, explain why relative wealth was not protective as it was seen in other settings (Huang and Manderson, 2005; Raso et al., 2005a). The slope and elevation of the village were used as proxies for the location and relief of the fields which are generally located nearby.

Previous research has indicated that the 0-1°C January isotherme limits the northern range of *O. hupensis* in otherwise suitable snail habitats in eastern China (Yang et al., 2005b). It remains to be seen if a corresponding critical value can be determined in the schistosome-endemic mountainous areas of western China, where a temperature-dependent limitation at a certain elevation would be expected. We found an inverse relationship between seroprevalence and altitude, but this could also be explained by steeper slopes and, consequently, fewer potentially suitable snail habitats at higher altitudes. We distinguished slopes above and below 2150 m. This altitude cut-off was motivated by geographical criteria;

it represents the upper delimitation of the slightly inclined plain areas in eastern Eryuan, rather than a temperature threshold.

As a whole, a picture emerges where the regional epidemiological situation of schistosomiasis japonica is governed by environmental factors determining the presence or absence of the intermediate host snail, whilst the local prevalence reflects demographic (occupation playing a key role) and socio-economic risk factors and the influence of sustained control efforts.

There are several shortcomings of our study that warrant discussion. The points offered here might also provide some directions for further research. Firstly, the availability of high-resolution RS data from China is still somewhat limited. For example, we were unable to derive detailed information on different land-use patterns, including irrigated agriculture, and common soil types and moisture. The availability of such environmental data might enhance the predictive power of spatially-explicit models. It is conceivable that the identification of agricultural land utilized by the farmers from each village, and its classification into different crop and irrigation types, as well as the determination of the non-agricultural population and their movements, could aid in further refining *S. japonicum* risk profiles. Secondly, models for the prediction of snail habitats, including the identification of critical isothermes governing *O. hupensis* populations in mountainous areas, should be developed and results incorporated in the prediction of human infection. The prediction of the snail habitats would also enable the determination of the distance between villages and potential snail habitats, and hence the inclusion of this covariate in future predictive models.

The national schistosomiasis control programme in China has led to the elimination of schistosomiasis japonica from five of the previously 12 endemic provinces and has achieved a significant reduction of the number of cases in the remaining endemic areas (Utzinger et al., 2005; Zhou et al., 2005). Repeated rounds of praziquantel, administered at a large scale, have not only reduced the prevalence of infection, but have also decreased the mean intensity of infection and morbidity, and had an impact on transmission (Guo et al., 2006). At present, most infections are light and, consequently, the widely used Kato-Katz technique has become unreliable to detect all active infections, particularly if only a single stool sample is examined (Yu et al., 1998; Wang et al., 2006). Nevertheless, a recent study concluded that the Kato-Katz technique is still recommended for screening of *S. japonicum* (Yu et al., 2007). However, more than a decade ago, the low prevalence of *S. japonicum* across large parts of China has led to the adoption of a two-pronged diagnostic approach, namely population screening by a

serological assay (e.g. indirect hemagglutination assay or ELISA), followed by microscopic examination of stool samples from seropositive individuals (Zhu, 2005).

The invasive nature of the screening test and the effort required to screen entire populations call for other means to promptly and reliably identify high-risk populations to target control efforts in a cost-effective manner. Snail surveys, including the determination of the *S. japonicum* infection status of recovered *O. hupensis*, is the traditional means to identify schistosome-endemic areas in China (Utzinger et al., 2005) and control efforts are focused on these snail-endemic areas. These surveys are labour intensive as the focal nature of *O. hupensis* requires a dense network of sampling sites and the snail infection rate is generally low. The development of spatially-explicit prediction models for the identification of potential snail habitats, employing remotely-sensed environmental data holds promise to focus ground surveys on those areas that are ecologically suitable for *O. hupensis* (Yang et al., 2005c). Prediction models could also help to identify even relatively small habitats that might have been overlooked thus far.

The prediction of the seroprevalence among humans could focus the control activities targeting humans on those areas where they are most needed. The use of serological screening methods in areas of low infection intensity after repeated rounds of chemotherapy has been advocated repeatedly (Maddison, 1987; Hoshino-Shimizu et al., 1992). Indeed, previous experiences from sero-epidemiological studies in areas of low endemicity highlight the advantages associated with the use of serological methods for surveillance, e.g. in the Philippines (Yogore et al., 1983), Kenya (Doenhoff et al., 1993) and Puerto Rico (Hillyer and Soler de Galanes, 1999). The use of the seroprevalence instead of the parasitological prevalence is justified on the following grounds. Firstly, the serostatus offers an idea on the infection pressure irrespective of recent control activities. Most people seroconvert shortly after the infection and about half of them remain seropositive 1-2 years after they have been parasitologically cured (Zhu, 2005). However, it should be noted that serological techniques do not reliably distinguish current from past infections. Secondly, the sensitivity of the Kato-Katz technique employed on a single stool sample is low, especially for light infections (Yu et al., 1998; Wang et al., 2006), and the collection of multiple stool samples from the same person during large-scale surveys is difficult due to logistical and financial constraints. Blood tests, on the other hand, are a standard means in large-scale surveys in China, as they are quick and relatively inexpensive (Zhu, 2005). Thirdly, an enhanced focus on humans has the advantage that at-risk populations might be identified and included into future control efforts, even if they do not live in the vicinity of snail areas, but are nonetheless at risk of infection at

more distant sites. Seropositives or even parasitologically-positive people can be found in areas that are considered non-endemic based on the traditional approach of first screening the local population of areas where infected snails are found by a serological method and subsequently assessing a single stool sample of the seropositives by the Kato-Katz technique. This was also shown in the present study (Steinmann et al., 2007). Fourthly, the continued success of chemotherapy-based morbidity control in China will lead to an ever growing share of low-intensity infections. However, it is unlikely that praziquantel alone will be sufficient to completely and permanently eliminate schistosomiasis from entire regions (Utzinger et al., 2003; Bergquist et al., 2005; King et al., 2006). Working with the serostatus rather than the parasitologically-confirmed infection could offer an alternative to overcome some of these challenges.

We conjecture that the seroprevalence can be regarded as an indicator of the epidemiological situation in the absence of chemotherapy. As long as transmission sites exist and people come into contact with them, there is seroconversion, even if ensuing disease is rare due to individual treatment or mass treatment campaigns. A large difference between the seroprevalence and the parasitological prevalence could indicate a prominence of treatment in the set of employed tools for control. If the difference is maintained, it could further mean that the discontinuation of chemotherapy-based control efforts will ultimately result in an increase of the parasitological prevalence up to the seroprevalence level. It could therefore be argued that the gap between the seroprevalence and the parasitological prevalence is a measure for the effectiveness and sustainability of control.

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10. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods

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10.1 Summary

Intestinal multiparasitism, the accuracy of different diagnostic techniques, and the influence of sampling effort were studied among 215 individuals in a Bulang village, Yunnan province, People's Republic of China. Behavioral, demographic and socioeconomic data were obtained by questionnaire. Multiple stool specimens were examined by the Kato-Katz, Koga agar plate, Baermann, and ether-concentration methods. Eight helminth and 7 protozoa species were diagnosed. The prevalence of each of the three main soil-transmitted helminths (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) exceeded 85%. *Blastocystis hominis* was the most prevalent intestinal protozoan (20.0%). Over 80% of the individuals harbored 3 or more intestinal parasites concurrently. The infection intensities were predominantly light for hookworm and *T. trichiura* but moderate for *A. lumbricoides*. Examination of 3 instead of 1 stool specimen increased the sensitivity of helminth diagnosis, most notably for hookworm. Intestinal multiparasitism is rampant in this rural part of Yunnan province and calls for control measures.

Keywords soil-transmitted helminths, intestinal protozoa, multiparasitism, diagnosis, sensitivity, People's Republic of China

10.2 Introduction

Multiparasitism is common among deprived populations, especially those living in developing countries.[1,2] Although this issue has been noted for decades,[3,4] contemporary epidemiologic investigations still focus on single species infections or groups of related parasites.[5] Cross-sectional surveys usually list prevalence data for different parasites separately but neglect to report the extent of multiple species parasite infections. Recently, the interest in the epidemiology of multiparasitism has grown, but the limitations put forward by Keiser and colleagues in 2002[6] still apply: (i) no single standardized diagnostic technique capable of detecting all intestinal, not to mention *all*, parasites with high sensitivity is available; (ii) most studies have focused on particular age groups; and (iii) little progress has been made in the attribution of specific symptoms out of the array of commonly encountered signs of morbidity to infections with particular parasites. Nevertheless, there is increasing awareness that new research is needed to deepen our understanding of multiparasitism, because even low-intensity infections with several helminths can cause significant morbidity.[2,7,8] Moreover, interactions between different parasites or common modes of transmission can result in notable associations between certain species.[6,9,10]

It is not feasible to screen entire populations for all potentially endemic parasites. However, concentrating on 1 or 2 kinds of biological samples, and subjecting these to a suite of diagnostic approaches has proven useful. In most studies focusing on multiparasitism, stool samples were screened for helminth infections. Only a few studies examined stool samples for helminths and intestinal protozoa concurrently,[6,11] and only rarely was complementary blood testing done for the concurrent diagnosis of malaria.[9]

Although the approach taken depends on the parasites to be investigated, the sensitivity of common diagnostic techniques can be enhanced by screening multiple samples of suitable biofluids.[12-15] Thus, the combination of different diagnostic approaches and multiple samples holds promise to reveal the 'true' extent of multiparasitism.[16]

The bulk of the published literature on intestinal multiparasitism stems from Africa.[6,9,17-19] Comparatively few data are available from Southeast Asia and the People's Republic of China, at least on PubMed.[11,20-22] During the first national sampling survey on human parasites in China, carried out between 1988 and1992, fecal samples of ~1.48 million individuals were screened by the Kato-Katz technique and Lugol-stained direct smears. Among those infected with at least 1 parasite, 43.3% were found to harbor 2-9 species concurrently.[23] The second national sampling survey, conducted between 2001 and 2004,

documented a significantly lower prevalence of common soil-transmitted helminths, but unfortunately no information is available on intestinal protozoa.[24] Regarding soil-transmitted helminthiasis, data from the 2 surveys suggest that the focus had shifted from south eastern to central and western China. It is not known whether the epidemiology of intestinal protozoa followed the same trend.

The objective of this study was to assess the extent of intestinal multiparasitism in a village in southern Yunnan province, China. For this purpose, multiple stool specimens were examined with a suite of diagnostic approaches for the presence of helminths and intestinal protozoa. The design of our study allowed the investigation of the performance of different diagnostic techniques, and to study the influence of sampling effort on the recorded prevalence of single and multiple species parasitic infections.

10.3 Materials and methods

10.3.1 Study site and selection of participants

The study was carried out in May 2006 in Nongyang, a settlement belonging to the administrative village of Manguo in Menghai county, Xishuangbanna prefecture, Yunnan province, China (geographic coordinates: 100.35° E longitude, 21.81° N latitude). The village and the selection of the study participants have been described elsewhere.[25,26] In brief, Nongyang is located 1350 m above sea level. Smallholder animal husbandry and subsistence farming are common livelihood practices. In addition, people are engaged in tea and sugarcane farming for cash. The village possesses the basic infrastructure that is typically found in mountain settlements in this part of China: power, telephone, supply of untreated water at household level, and a community latrine. However, no family has its own latrine.

The local health authorities informed the village leaders about the aims and procedures of the study. After receipt of consent from local authorities and the village registry listing the 150-plus local households, all members of the 78 families with uneven registration numbers were enrolled in cohorts of 20-30 families per week.

10.3.2 Field and laboratory procedures

Details on field and laboratory procedures have been described before.[25,26] In brief, we administered pretested individual and household-level questionnaires to obtain data on demography, occupation, behavior, living conditions, and agricultural and household asset ownership. In parallel, prelabeled stool-collection containers were handed out to every

participant. Stool specimens were collected every morning, and new containers were distributed with the goal to obtain 3 specimens per individual.

The stool specimens were brought to the laboratory and processed within a maximum of 12 hours post-collection. First, the presence of *Taenia* spp. proglottids was recorded. Second, a single Kato-Katz thick smear was prepared using 41.7-mg punched plastic templates.[27] Helminth eggs were enumerated on a per-species basis within 1 hour under a light microscope. Third, about 10 g of stool was used to perform the Baermann test.[28] Fourth, 1-2 g of stool was placed on an agar plate for evaluation according to the Koga method.[29] The latter 2 tests were mainly employed for diagnosis of *Strongyloides stercoralis*, but hookworm larvae can also be detected on the Koga agar plates.[30] Finally, for each individual, 1-2 g of stool was thoroughly mixed with 10 ml sodium acetate-acetic acid-formaline (SAF) solution and shipped to a Swiss reference laboratory for subsequent semi-quantitative examination for helminth eggs and intestinal protozoa, using a standardized SAF-ether-concentration method.[31]

10.3.3 Statistical analysis

Data were double-entered and cross-checked using EpiData version 3.0 (EpiData Association; Odense, Denmark). After a number of internal consistency checks had been performed, the data were transferred to STATA version 9.2 (StataCorp.; College Station, TX), and all statistical analyses were done with STATA.

The final cohort comprised those individuals who had ≥2 Kato-Katz thick smear readings plus ≥2 Koga agar plate test results plus the result from the SAF-ether-concentration method. In addition, the Baermann test was required for the evaluation of *S. stercoralis*.

Prevalence of individual parasites and multiparasitism were determined on the basis of combined results from the different diagnostic methods. Infection intensity for helminths was calculated using egg counts from the Kato-Katz thick smears only, with stratification into light, moderate, and heavy infections according to cutoff values put forward by the World Health Organization (WHO).[32] Associations between prevalence or infection intensity classes and sex or age were investigated using Pearson's χ^2-statistics.

The performance of the diagnostic techniques was assessed based on the assumption that the combined results of all tests accurately reflected the true infection status. Individual tests were compared with this diagnostic 'gold' standard and among each other by exploring the differences between the respective proportions. Multiple logistic regression with stepwise manual backward elimination of variables at a level of $P = 0.15$ according to the likelihood-

ratio test (LRT) was used to test the associations of individual parasites with the remaining parasites, adjusted for sex and age. A family-level random effect was introduced to account for clustering of infections within families. The 'true' prevalence of *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura* was calculated based on the multiple Kato-Katz thick smear readings and according to a mathematical model described elsewhere.[14] This model has been used before to estimate the 'true' prevalence of soil-transmitted helminth infections[33] and *S. stercoralis* in the village studied here.[26]

10.3.4 Ethical considerations and treatment

The study was approved by the institutional review boards of the Swiss Tropical Institute (Basel, Switzerland) and the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, China). Authorities of the local medical services informed village leaders about the study procedures. After their consent to perform the study was obtained, field workers visited the homes of the selected families where detailed information about the study, including potential risks and benefits, was provided. Arising questions were answered. Voluntary participation and the option to quit the study at any moment without further obligation or negative consequence were emphasized. Written confirmation that full information had been provided and individual participation was voluntary (informed consent) was obtained from the head of each participating household or a literate substitute designated by the head of the family.

At the completion of the study and in accordance with the local treatment policies, health personnel of the responsible parasitic diseases control station in Menghai offered free treatment with the compound mebendazole (mebendazole 100 mg/tablet + levamisole hydrochloride 25 mg/tablet, 2 tablets per day for 3 consecutive days) to all individuals aged ≥5 years in Nongyang village.

10.4 Results

Compliance and demographics

Seventy-one families with 283 individuals were present at the time of the survey, all of whom agreed to participate. Figure 10.1 shows how many individuals complied with multiple stool specimen submission, allowing different diagnostic methods to be performed. Overall, 251 individuals (88.7%) submitted at least 1 sufficiently voluminous stool sample to carry out all but the Baermann test. Complete datasets with 2 or 3 stool samples were obtained from 215

(76.0%; final cohort) and 177 (62.5%) participants, respectively. The full range of diagnostic tests, including the Baermann method, could be performed for 234 (≥1 sample, 82.7%), 180 (≥2 samples, 63.6%) and 128 individuals (3 samples, 45.2%), respectively.

Females represented 52.6% of the final cohort. The age of the participants ranged from 4 to 84 years with a mean age of 29 years and proportions of 11.2%, 10.7%, 25.6%, 28.8% and 23.7% for the age groups 4-10, 10-14, 15-24, 25-39 and ≥40 years, respectively. There was no significant difference by sex among the age groups ($\chi^2 = 6.39$, degree of freedom (d.f.) = 4, $P = 0.172$) and the age and sex distribution of the final cohort was similar to that of all 283 study participants (both $P > 0.05$).

10.4.1 Parasitic infections and multiparasitism

Screening of at least 2 stool specimens per individual using 4 different diagnostic tests resulted in detection of 15 intestinal parasite species; 8 helminths and 7 protozoa (Table 10.1). Very high prevalences were recorded for *A. lumbricoides* (92.6%), hookworm (88.8%), and *T. trichiura* (88.8%). The highest prevalence of *A. lumbricoides*, hookworm and *T. trichiura* infections were found among individuals of age between 10 and 24 years, but only *T. trichiura* prevalence differed significantly among age groups ($\chi^2 = 19.86$, d.f. = 4, $P = 0.001$). The prevalence of *S. stercoralis* was 11.7%; detailed results pertaining to this parasite have been presented elsewhere.[26] The remaining helminths identified were *Enterobius vermicularis* (7.4%), *Taenia* spp. (5.1%), *Dicrocoelium dendriticum* (1.4%), and *Fasciolopsis buski* (0.5%). *Taenia* spp. infections were found only in males ($P < 0.001$), among ≥15-year-olds, and steadily, albeit not significantly, increased with age ($\chi^2 = 7.11$, d.f. = 4, $P = 0.119$).

The most common intestinal protozoan parasite was *Blastocystis hominis* (20.0%), followed by *Endolimax nana* (6.1%), *Entamoeba coli* (3.7%), *Iodamoeba bütschlii* (2.3%), *Giardia intestinalis* (1.9%), *Entamoeba hartmanni* (1.4%), and *Entamoeba histolytica/Entamoeba dispar* (0.5%). *G. intestinalis* was found only in individuals under age 25 years ($P = 0.109$), whereas *E. nana* was not found among participants younger than 15 years ($\chi^2 = 9.68$, $P = 0.046$).

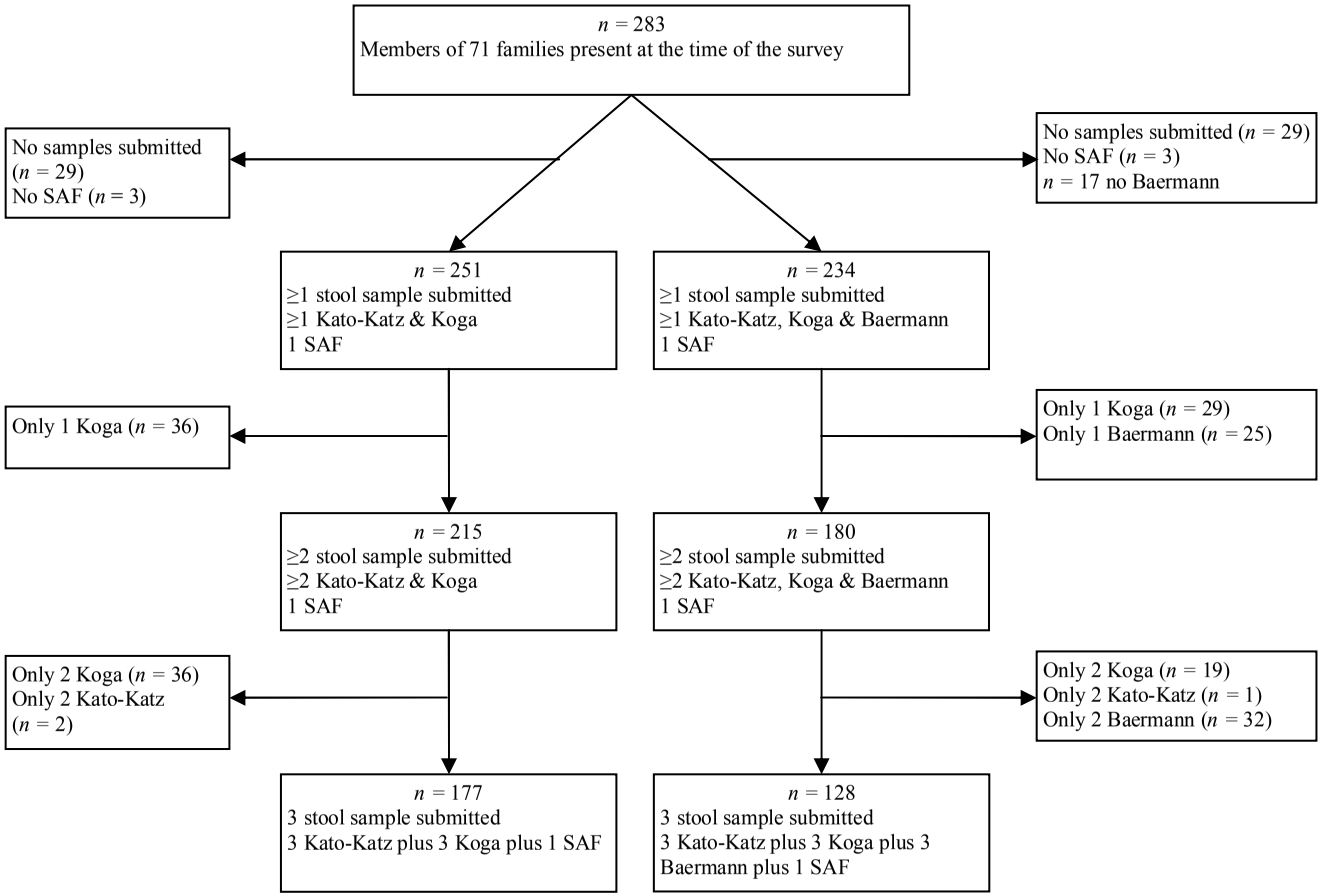

Figure 10.1 Compliance and number of diagnostic results among 283 inhabitants of Nongyang village in Yunnan province, China, who were invited to submit multiple stool specimens for screening by the Kato-Katz, Koga agar plate, Baermann and SAF-ether-concentration technique (only 1 sample for the latter).

Table 10.1 Total prevalence of helminths and intestinal protozoa among 215 (*S. stercoralis*: 180) study participants from Nongyang village in Yunnan province, China, after screening of ≥2 stool samples by the Kato-Katz, Baermann, Koga agar plate, and a SAF-ether-concentration method (only 1 sample/person), stratified by sex and age group

Parasite	Prevalence in % (n)	95% CI[a]	Pathogenic	Sex				Age (years)						
				Female	Male	χ^2	*P*-value[b]	4-9	10-14	15-24	25-39	>39	χ^2	*P*-value[b]
Helminths														
Ascaris lumbricoides	92.6 (199)	89.0-96.1	Yes	95.6	89.2	3.15	0.076	95.0	95.2	96.4	91.9	87.5	3.72	0.445
Trichuris trichiura	88.8 (191)	84.6-93.1	Yes	91.2	86.3	1.29	0.257	90.0	100	92.7	95.2	73.2	19.86	0.001
Hookworm	88.8 (191)	84.6-93.1	Yes	88.5	89.2	0.03	0.867	75.0	95.2	96.4	88.7	83.9	9.35	0.053
Strongyloides stercoralis	11.7 (21)	6.9-16.4	Yes	6.1	18.3	6.42	0.011	0	0	19.6	9.4	15.9	8.70	0.069
Enterobius vermicularis	7.4 (16)	3.9-11.0	Yes	8.9	5.9	0.69	0.408	5.0	0	8.9	8.1	8.9	2.26	0.689
Taenia spp.	5.1 (11)	2.1-8.1	Yes	0	10.8	12.84	<0.001	0	0	1.8	6.5	10.7	7.11	0.119
Dicrocoelium dendriticum	1.4 (3)	0-3.0	Yes	0.9	2.0	n.a.	0.605[c]	0	0	0	3.2	1.8	n.a.	0.881[c]
Fasciolopsis buski	0.5 (1)	0-1.4	Yes	0	1.0	n.a.	0.474[c]	0	0	0	0	1.8	n.a.	0.712[c]
Protozoa														
Blastocystis hominis	20.0 (43)	14.6-25.4	Uncertain	23.0	16.7	1.35	0.246	20.0	19.1	21.4	24.2	14.3	1.91	0.753
Endolimax nana	6.1 (13)	2.8-9.3	No	8.9	2.9	3.29	0.070	0	0	7.1	12.9	1.8	9.68	0.046
Entamoeba coli	3.7 (8)	1.2-6.3	No	5.3	2.0	1.68	0.195	10.0	0	5.4	3.2	1.8	4.06	0.398
Iodamoeba bütschlii	2.3 (5)	0-4.4	No	2.7	2.0	0.11	0.736	0	0	5.4	1.6	1.8	3.45	0.485
Giardia intestinalis	1.9 (4)	0-3.7	Yes	1.8	2.0	n.a.	1.000[c]	5.0	4.8	3.6	0	0	n.a.	0.109[c]
Entamoeba hartmanni	1.4 (3)	0-3.0	No	1.8	1.0	n.a.	1.000[c]	0	0	3.6	1.6	0	n.a.	0.751[c]
Entamoeba histolytica/ E. dispar	0.5 (1)	0-1.4	Yes/No	0	1.0	n.a.	0.474[c]	5.0	0	0	0	0	n.a.	0.093[c]

[a] CI, confidence interval; n.a., not applicable
[b] Pearson's χ^2-test
[c] Fisher's exact test

Multiparasitism was very common; not a single individual was uninfected, and we identified up to 6 parasite species per individual; 1-5 helminths and as many as 3 intestinal protozoa. Almost half of the study participants harbored 3 parasite species concurrently (45.1%), and another 26.6% were infected with 4 species. Single-species helminth infections were rare (4.2%), 3 helminth species were diagnosed in 62.3% of the participants, and 5 species in 1.4%. On the other hand, no intestinal protozoa were diagnosed among three-quarters of the population sample, whereas triple species protozoan infections were diagnosed in 3.3% (Figure 10.2). No significant differences were found between the number of helminth, protozoa or general parasite species per person and sex or age, but there was a tendency for multipl-parasite species infections among elder participants; concurrent infections by 3 different protozoa, 5 helminth species, or 6 intestinal parasites of any kind were found only in participants ≥15 years (data not shown).

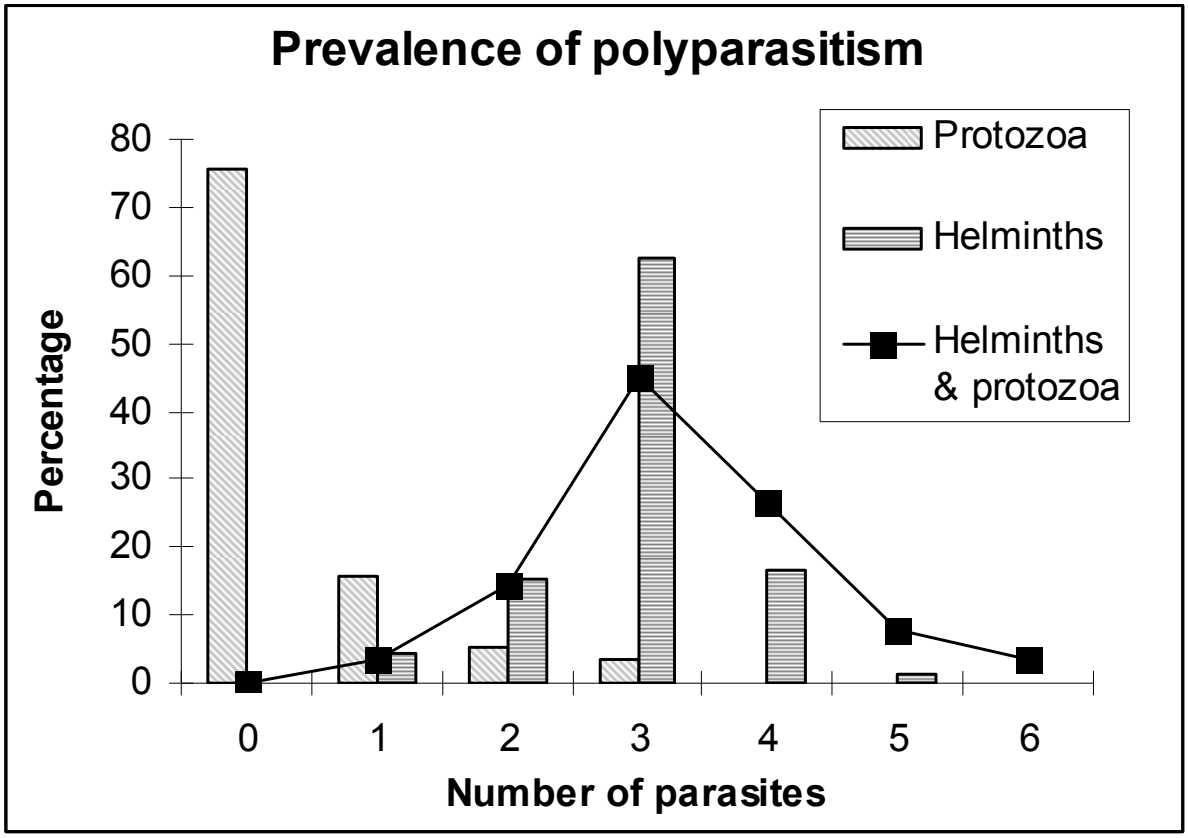

Figure 10.2 Prevalence of multiple species parasite infections among 215 study participants from Nongyang village in Yunnan province, China.

10.4.2 Helminth infection intensities

Because the Kato-Katz method is quantitative, infection intensities could be calculated for the major soil-transmitted helminths. The geometric mean number of eggs per gram stool (epg) among the infected was 7525 epg (95% confidence interval (CI) = 5850-9680 epg) for *A. lumbricoides*, 137 epg for hookworm (95% CI = 114-164 epg), and 121 epg (95% CI = 103-144 epg) for *T. trichiura*. The infection intensities, stratified by sex and age group, are shown in Figure 10.3 and Figure 10.4. The majority of *A. lumbricoides* infections were of moderate intensity (58.5%). Light and heavy infections made up 31.3% and 10.2% of all cases, respectively. Hookworm and *T. trichiura* infections were primarily of light intensity; 98.8% and 97.8%, respectively. There was no significant difference in the infection intensity of any parasite between males and females (all $P > 0.05$), but moderate-intensity hookworm infections were limited to males ≥25 years old. The *A. lumbricoides* and *T. trichiura* infection intensities varied significantly between age groups ($\chi^2 = 22.88$, d.f. = 12, $P = 0.029$ and $\chi^2 = 30.30$, d.f. = 8, $P < 0.001$, respectively), whereas the hookworm infection intensity showed borderline significance ($\chi^2 = 15.32$, d.f. = 8, $P = 0.053$). Heavy *A. lumbricoides* infections were mainly found among children of age 4-9 years (30% of this subpopulation *versus* 7.2% in all other age groups).

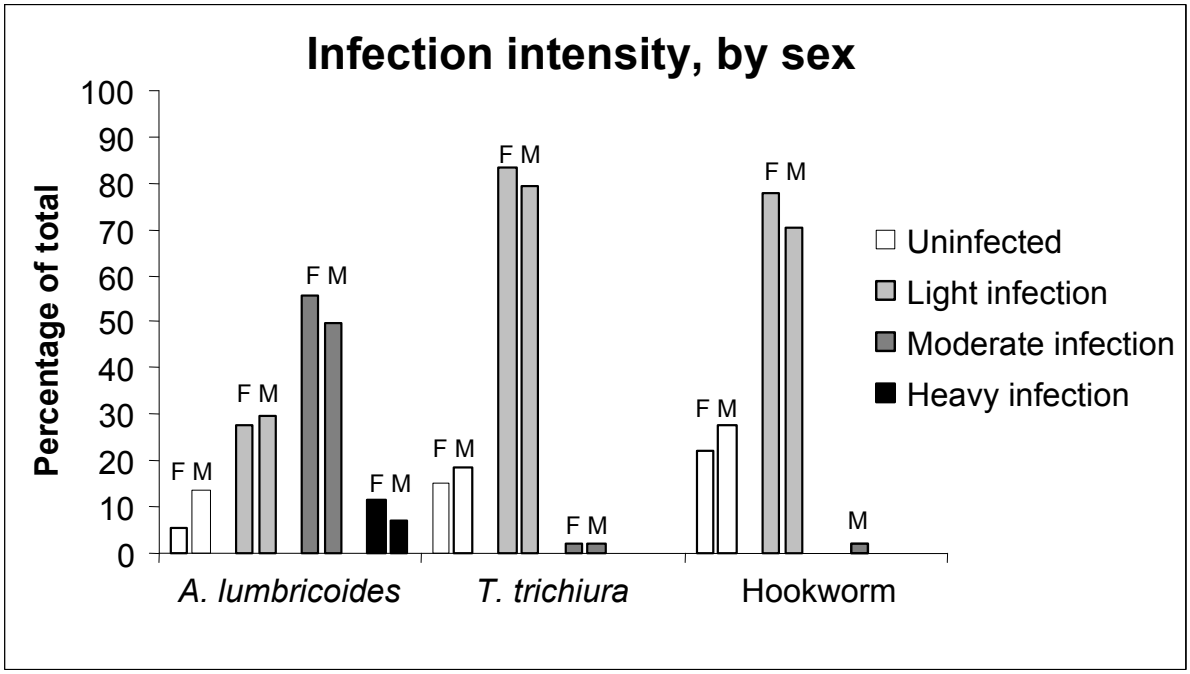

Figure 10.3 Infection intensity of *A. lumbricoides*, *T. trichiura*, and hookworm, stratified by sex, among 215 study participants from Nongyang village in Yunnan province, China. Mean of 2-3 stool samples assessed by the Kato-Katz method and classified according to WHO guidelines (F, females; M, males)

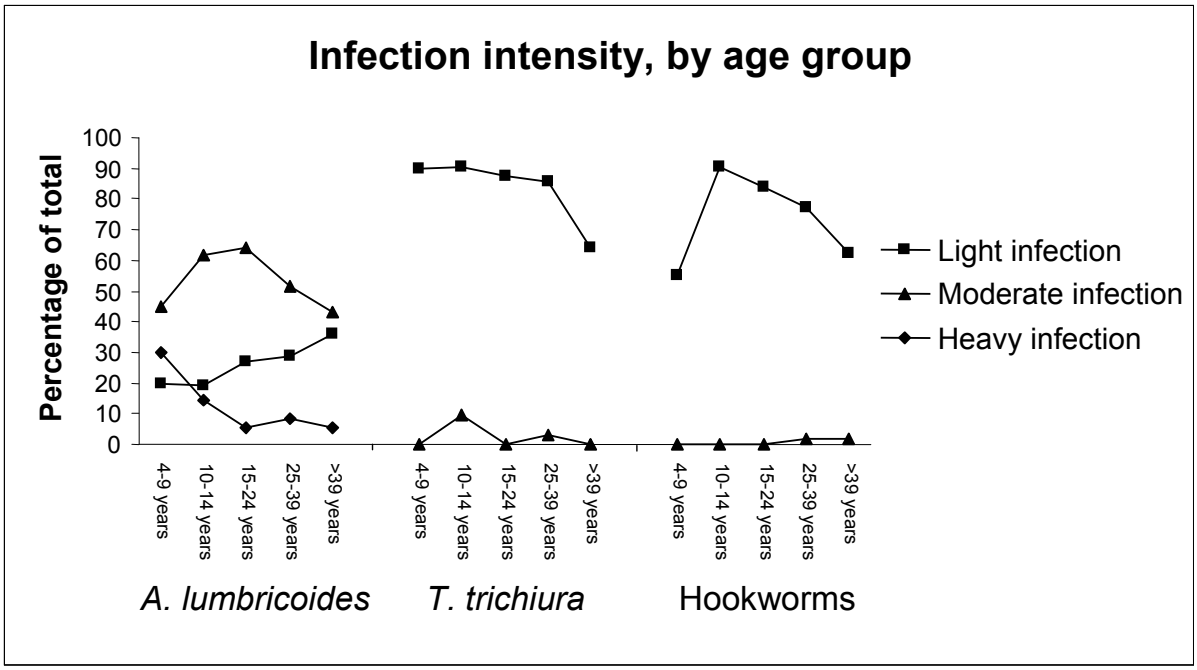

Figure 10.4 Age group-stratified infection intensity of *A. lumbricoides*, *T. trichiura*, and hookworm among 215 study participants from Nongyang village in Yunnan province, China. Mean of 2-3 stool samples assessed by the Kato-Katz method and classified according to WHO guidelines.

10.4.3 Parasite associations

Table 10.2 shows the association of each parasite with the remaining ones, adjusted for sex and age. Study participants harboring *A. lumbricoides* were often co-infected with *T. trichiura* (odds ratio (OR) = 5.65, $P = 0.007$). *B. hominis* was significantly associated with *E. vermicularis* (OR = 3.78, $P = 0.036$) and the protozoa *E. nana* and *E. coli.* It was also often found in individuals with *T. trichiura* (OR = 7.18), but this association did not reach conventional statistical significance ($P = 0.066$). Some nonpathogenic intestinal protozoa were less often found in people with particular helminth infections. *Endolimax nana*, for example, was less prevalent among people with *A. lumbricoides* infections (OR = 0.17, $P = 0.045$), and hookworms were less often found among participants with an *I. bütschlii* infection (OR = 0.08, $P = 0.100$). However, the latter association did not reach standard statistical significance.

Table 10.2 Stepwise multiple logistic regression using backward elimination at a level of *P* = 0.15 to investigate associations between individual parasites and the remaining parasites, sex, and age group among 215 study participants from Nongyang village in Yunnan province, China

Parasite	Associations (*P* <0.15)	OR (95% CI)	*P*-value
Helminths			
Ascaris lumbricoides	*Trichuris trichiura*	5.65 (1.61-19.80)	0.007
	Endolimax nana	0.17 (0.03-0.96)	0.045
	Iodamoeba bütschlii	0.15 (0.02-1.21)	0.075
	Sex: female	1.00	
	male	0.29 (0.08-1.00)	0.050
Trichuris trichiura	*Ascaris lumbricoides*	4.34 (1.14-16.50)	0.031
	Blastocystis hominis	7.18 (0.88-58.94)	0.066
	Age	0.55 (0.35-0.88)	0.012
Hookworm	–	–	
Strongyloides stercoralis	Sex: female	1.00	
	male	3.04 (1.20-7.70)	0.019
Enterobius vermicularis	*Blastocystis hominis*	2.65 (0.87-8.02)	0.085
Taenia spp.	–	–	
Protozoa			
Blastocystis hominis	*Enterobius vermicularis*	3.78 (1.09-13.11)	0.036
	Endolimax nana	15.57 (3.02-80.36)	0.001
	Entamoeba coli	14.66 (2.15-100.09)	0.006
	Iodamoeba bütschlii	11.98 (0.77-187.45)	0.077
Endolimax nana	*Blastocystis hominis*	13.40 (3.34-53.79)	<0.001
	Iodamoeba bütschlii	11.22 (1.31-96.28)	0.028
Entamoeba coli	*Blastocystis hominis*	13.86 (2.68-71.68)	0.002
Iodamoeba bütschlii	Hookworm	0.08 (<0.0-1.62)	0.100
	Blastocystis hominis	10.37 (0.70-154.54)	0.090
	Endolimax nana	18.28 (1.50-223.44)	0.023

CI, confidence interval; OR, odds ratio

10.4.4 Diagnostic performance of different techniques

Table 10.3 shows the performance of individual diagnostic techniques, including comparison with our designated 'gold' standard obtained from the pooled results of at least 2 methods for *A. lumbricoides*, hookworm, *T. trichiura*, and *S. stercoralis*, with findings for the latter parasite presented elsewhere.[26] The analysis of 2-3 stool samples with the Kato-Katz technique was slightly, but not significantly, more sensitive for *A. lumbricoides* (98.0%, 95% CI = 94.9-99.5%), *T. trichiura* (93.7%, 95% CI = 89.3-96.7%), and hookworm infection (84.8%, 95% CI = 78.9-89.6%) than the analysis of a single SAF-conserved sample by the ether-concentration method (*A. lumbricoides*: 93.0%, 95% CI = 88.5-96.1%; *T. trichiura*: 89.5%, 95% CI = 84.3-93.5%; hookworms: 77.5%, 95% CI = 70.9-83.2%). For the diagnosis of hookworm infection, the most sensitive method was the Koga agar plate technique. It detected 90.1% (95% CI = 84.9-93.9%) of all infections, significantly more than the SAF-ether-concentration method (77.5%, 95% CI = 70.9-83.2%; *P* = 0.008). The use of just 1

diagnostic method significantly underestimated the 'true' prevalence for all parasite-diagnostic technique combinations, except for the diagnosis of *A. lumbricoides* and *T. trichiura* by the Kato-Katz technique.

The analysis of 3 instead of a single stool specimen considerably increased the sensitivity of the diagnostic tools (Table 10.4). The difference was most pronounced for diagnosis of hookworm infections. The measured prevalence increased from 43.6% (first sample) to 76.6% (third sample) and from 54.2% to 81.0% for the Kato-Katz and Koga agar plate methods, respectively. The corresponding sensitivity of a single test was 56.9% and 66.9%, respectively. Table 10.4 also lists the frequency of positive test results among the 3 samples collected from every individual and the derived 'true' prevalences as well as sensitivities of the analysis of single or triple samples. The model predicted only marginally higher 'true' prevalences than the actually recorded ones and, consequently, high sensitivities for the analysis of 3 samples. However, the estimated sensitivity of analyzing a single sample was considerably above the measured value for the diagnosis of hookworm infection by the Kato-Katz technique (measured: 56.9%; predicted: 70.3%).

Table 10.3 Performance of individual techniques for the diagnosis of soil-transmitted helminth infections compared with our sesignated diagnostic 'gold' standard among 215 individuals from Nongyang village in Yunnan province, China

Parasite	*A. lumbricoides*			*T. trichiura*			Hookworms			
Test	Kato-Katz (2-3 samples)	SAF-ether-concentration (1 sample)	'Gold' standard ('true' prevalence)[b]	Kato-Katz (2-3 samples)	SAF-ether-concentration (1 sample)	'Gold' standard ('true' prevalence)[b]	Kato-Katz (2-3 samples)	SAF-ether-concentration (1 sample)	Koga agar plate (2-3 samples)	'Gold' standard ('true' prevalence)[b]
Prevalence (%) 95% CI[a]	90.7 (86.8-94.6)	86.0 (81.4-90.7)	92.6 (89.1-96.1)	83.3 (78.3-88.2)	79.5 (74.1-84.9)	88.8 (84.6-93.0)	75.3 (69.6-81.1)	68.8 (62.6-75.0)	80.0 (74.7-85.3)	88.8 (84.6-93.0)
Sensitivity (%) 95% CI[a]	98.0 (94.9-99.5)	93.0 (88.5-96.1)	100	93.7 (89.3-96.7)	89.5 (84.3-93.5)	100	84.8 (78.9-89.6)	77.5 (70.9-83.2)	90.1 (84.9-93.9)	100
Difference[c] 95% CI[a]	1.9 (-3.4-7.1)	6.5 (0.7-12.3)		5.6 (-0.9-12.1)	9.3 (2.5-16.1)		13.4 (6.4-20.6)	20.0 (12.5-27.5)	8.8 (2.0-15.6)	
P-value	0.486	0.029		0.095	0.008		< 0.001	< 0.001	0.012	
Negative predictive value (%) 95% CI[a]	80.0 (56.3-94.3)	53.3 (34.3-71.7)		66.7 (49.0-81.4)	54.6 (38.9-69.6)		45.3 (31.6-59.6)	35.8 (24.5-48.5)	55.8 (39.9-70.9)	

[a] CI, confidence interval
[b] "Gold" standard: *A. lumbricoides*, *T. trichiura*: combined results of Kato-Katz plus SAF-ether-concentration; hookworm: combined results of Kato-Katz plus SAF-ether-concentration plus Koga agar plate
[c] Difference between measured prevalence and 'true' prevalence

Table 10.4 Influence of the collection and analysis of multiple stool samples on the observed prevalence, estimated 'true' prevalence and sensitivity of the diagnostic technique; samples were collected among inhabitants of Nongyang village in Yunnan province, China

Feature	Kato-Katz									Koga agar plate		
	A. lumbricoides			*T. trichiura*			Hookworm			Hookworm		
	No.	%	Sens.[a]	No.	%	Sens.[a]	No.	%	Sens.[a]	No.	%	Sens.[a]
Number of samples analyzed	188	100		188	100		188	100		179	100	
Cumulative prevalence after analysis of												
1st stool sample	156	83.0	91.8	114	60.6	71.3	82	43.6	56.9	97	54.2	66.9
2nd stool sample	167	88.8	98.2	147	78.2	91.9	123	65.4	85.4	132	73.7	91.0
3rd stool sample	170	90.4	100	160	85.1	100	144	76.6	100	145	81.0	100
Helminth eggs/larvae detected in												
1 stool sample	10	5.9		27	16.9		36	25.0		40	27.6	
2 stool samples	11	6.5		51	31.9		48	33.3		51	35.2	
3 stool samples of positive participants	149	87.6		82	51.2		60	41.7		54	37.2	
Estimated prevalence[b]		90.5±4.3			86.1±5.3			78.7±6.4			83.9±6.2	
Sensitivity (single sample)		93.9±2.1			77.2±4.1			70.3±4.8			67.5±5.0	
Sensitivity (3 samples)[c]		100			98.8			97.4			96.6	

[a] Sens., sensitivity; "gold" standard: 3 samples analyzed with the respective method
[b] Prevalence and performance indicators of diagnostic tests estimated using a mathematical model described by Marti & Koella (1993)
[c] Measured *versus* predicted prevalence

10.5 Discussion

We collected up to 3 stool specimens from 215 individuals living in a village in the southern part of Yunnan province, China. The stool samples were screened for helminths and intestinal protozoa using 4 different diagnostic methods, i.e., Kato-Katz, Baermann, Koga agar plate, and ether-concentration after conservation of the sample in SAF solution for 3 months. We found prevalences in excess of 85% for the 3 main soil-transmitted helminths. Five additional helminth species were recorded at prevalences ranging from 0.5% (*F. buski*) to 11.7% (*S. stercoralis*). Interestingly, much lower prevalences were recorded for intestinal protozoa than for the 3 main soil-transmitted helminths, the most common one being *B. hominis,* which parasitized one-fifth of the study cohort. Consequently, multiple species helminth infections were the norm, but multiple intestinal protozoa infections were the exception. Our study population harbored up to 6 different parasites concurrently and among those who submitted at least 2 stool samples we found none who was free of an infection. The infection intensities were predominantly light for hookworm and *T. trichiura* but moderate for *A. lumbricoides*. For the latter parasite, we also noted heavy infections. The maximum mean epg value was 191,700, counted in the stool samples of a 28-year-old female.

The low prevalence of pathogenic intestinal protozoa in this part of the world has been noted before[20] but has not attracted wide attention, and we are not aware of any explanation for this observation. Giboda and colleagues[20] also pointed out a high prevalence of multiple species helminth infections. Country-wide surveys among schoolchildren in neighboring Myanmar and Lao PDR employing the Kato-Katz technique on a single stool sample reported very high prevalences of soil-transmitted helminths. Interestingly, the survey in Myanmar[34] noted the lowest prevalences in the hilly area, which includes ecological zones that are similar to our study area. In Lao PDR, the highest prevalences of soil-transmitted helminths were reported from the northern provinces,[35] which share many eco-epidemiologic characteristics with Xishuangbanna prefecture in China. It should be noted that the environmental and epidemiologic conditions of southern Yunnan are distinct from other parts of the province. Hence, generalizations are difficult. This can be illustrated by comparing the results from the current study to data obtained during a parasitologic survey in Eryuan county in north-western Yunnan. The Eryuan study included 35 villages situated at elevations between 1750 m and 2700 m, and we found an overall prevalence of helminth infections of 20.5%.[36]

There are several limitations to our study. Together with issues already covered before,[26] we offer the following points. An important gap is the infection status of children below the

age of 4 years. Families were reluctant to enroll their young children for the study and it is difficult to obtain large-enough stool samples from this age group. Therefore, we are not in a position to conjecture whether multiparasitism is an issue among infants and preschoolers, and hence the age of infection for intestinal parasites remains to be investigated. Further, it could be argued that we overestimated the 'true' infection intensities but underestimated prevalences because of false-negative results from people with low-intensity infections for which the Kato-Katz technique lacks sensitivity. However, we are confident that this was not a major issue because the measured prevalence differed only slightly from the estimated 'true' values, and the latter were somewhat lower than the combined results from all techniques. It is also conceivable that we slightly overestimated the sensitivity of the evaluated diagnostic methods because no absolute diagnostic 'gold' standard was available. Instead, we considered the combined results from the different approaches as our reference. The very high prevalence of all considered parasites does not leave room for large differences between our assumed and the 'true gold' standard and therefore renders it unlikely that overestimation of the performance of single techniques was a major issue. The prevalence of *E. vermicularis* was certainly underestimated because we did not use the standard diagnostic approach for this helminth, i.e. the Scotch-tape method.[28] Finally, the absolute strengths of the identified associations between parasites should not be overvalued because they result from multiple analyses.

It is widely acknowledged that the analysis of a single stool sample by only one technique results in a considerable number of false-negative results.[15,16,37-39] The benefits associated with analyzing multiple stool samples by different diagnostic approaches have been confirmed in our study for helminth infections. For protozoa, however, we tested a single stool sample by only 1 technique. Therefore, we anticipate that the prevalence of intestinal protozoa has been underestimated. This can be illustrated for *B. hominis*, for which we recorded a prevalence of 20.0%, whereas the screening of the same samples by a more sensitive culture method resulted in a prevalence of 32.6%.[25] Similar or even larger discrepancies between the measured and the 'true' prevalence must be assumed for other intestinal protozoa. Our results indicate that under the prevailing conditions, the screening of 3 stool samples by 2-3 methods is likely to detect most helminth infections. Becaise we used only the SAF-ether-concentration technique for the diagnosis of intestinal protozoa, we are unable to comment whether it is sufficient to analyze 3 stool samples by this technique or if multiple techniques should be used.

The reliable diagnosis of hookworm infections is more difficult compared with *A. lumbricoides* and *T. trichiura*, particularly if only 1 stool sample is available or a several-hour time delay occurred between stool production, sample collection, and analysis.[40,41] The results of 3 methods (Kato-Katz, SAF-ether-concentration, and Koga agar plate) applied to 2-3 stool samples were available to us, and we observed individual sensitivities of 77.5-90.1% when compared with the overall result. Interestingly, culture on agar plates exhibited the highest sensitivity, a result that could not be expected based on the limited prior experience with this method.[30] It should further be noted that we tested 3 stool samples by the Koga agar plate and the Kato-Katz techniques, but only 1 SAF-conserved sample by ether-concentration. Our result strongly suggests that screening for *S. stercoralis* by the Koga agar plate method has additional benefits for the detection of hookworm infections. Employing this technique would also allow differentiation of hookworm species, i.e. *Ancylostoma duodenale* and *Necator americanus*, if desired.

Sensitive diagnostic methods that reliably detect the presence of infections of any intensity are not only important for individual diagnosis but should also be consistently used in epidemiologic studies and surveys at population level. They are particularly valuable for baseline studies and evaluation of treatment interventions. At baseline, it is important to know the 'true' prevalence of all parasites in the population, including their distribution among population subgroups, to tailor control programs to the local conditions, thereby enhancing the cost-effectiveness of the intervention. At follow-up, the use of sensitive diagnostic tools is paramount for the detection of the predominantly low-intensity infections among individuals who had not been cured completely.[42,43] We speculate that, currently, the prevalence at baseline is often underestimated whereas the efficacy of drug treatment is overestimated because the relative proportion of difficult-to-detect low-intensity infections is likely to be higher after chemotherapeutic interventions.[32]

Periodic treatment of millions of people with anthelminthic compounds[44-46] and improved socioeconomic conditions[47] have reduced the mean infection intensity in many parts of the world. Collection of multiple stool samples and analysis by different methods is one option to improve the sensitivity of common diagnostic tools. Another approach would be development of novel diagnostic techniques. Little emphasis has been placed on the development of new diagnostics for intestinal parasites. However, a notable exception is the FLOTAC technique.[48] This method can diagnose intestinal protozoa and helminth infections concurrently with high sensitivity,[49] and, in a recent study carried out in Côte d'Ivoire, its performance for hookworm diagnosis has been shown to be superior to the Kato-Katz and a standard SAF-ether-

concentration method.[50] If the high sensitivity of this novel approach can be confirmed for other helminths and for intestinal protozoa, then it will offer a new means for comprehensive assessment of intestinal multiparasitism.

The increasing recognition of the common phenomenon of polyparasitism has yet to trigger additional research on how this influences treatment. While it is true that common anthelminthic drugs are active against a range of helminth species[51] and often exhibit some activity against additional parasites, there is no single drug to treat the full range of intestinal helminths. For intestinal protozoa, the situation is similar.[52] WHO recommends preventive chemotherapy without prior diagnosis in areas of high endemicity.[32,53] Clearly, non-target parasites will be cotreated over the course of these campaigns, and it is possible that parasites are among them against which the drugs have never been tested. The practical evidence from the field suggests that most drugs do not exhibit high levels of adverse events, even if they are used in a context where multiparasitism has not been evaluated and where parasites other than the target species are likely to be present.[54] There are exceptions though, e.g., the administration of praziquantel to people suffering from cysticercosis.[55] Another area of concern is the development of resistance in nontarget parasites that could result from subtherapeutic drug concentrations. We are engaged in additional studies to investigate possible cross-benefits of chemotherapy resulting from the activity of anthelminthic drugs to nontarget parasites and we study the influence of polyparasitism on treatment outcomes. Finally, the results reported herein call for urgent interventions to reduce the burden of soil-transmitted helminthiasis, and the effect of regular administration of safe and efficacious anthelminthic drugs should be monitored.

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11. Occurrence of *Strongyloides stercoralis* in Yunnan province, China, and comparison of diagnostic methods

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11.1 Abstract

Background *Strongyloides stercoralis* is a neglected soil-transmitted helminth species, and there is a lack of parasitologic and epidemiologic data pertaining to this parasite in China and elsewhere. We studied the local occurrence of *S. stercoralis* in a village in Yunnan province, China, and comparatively assessed the performance of different diagnostic methods.

Methodology/principal findings Multiple stool samples from a random population sample were subjected to the Kato-Katz method, an ether-concentration technique, the Koga agar plate method, and the Baermann technique. Among 180 participants who submitted at least 2 stool samples, we found a *S. stercoralis* prevalence of 11.7%. Males had a significantly higher prevalence than females (18.3% *versus* 6.1%, $P = 0.011$), and infections were absent in individuals <15 years of age. Infections were only detected by the Baermann (highest sensitivity) and the Koga agar plate method, but neither with the Kato-Katz nor an ether-concentration technique. The examination of 3 stool samples rather than a single one resulted in the detection of 62% and 100% more infections when employing the Koga agar plate and the Baermann technique, respectively. The use of a mathematical model revealed a 'true' *S. stercoralis* prevalence in the current setting of up to 16.3%.

Conclusions/significance We conclude that *S. stercoralis* is endemic in the southern part of Yunnan province and that differential diagnosis and integrated control of intestinal helminth infections needs more pointed emphasis in rural China.

Keywords: *Strongyloides stercoralis*, epidemiology, diagnosis, Baermann technique, Koga agar plate method, Kato-Katz technique, ether-concentration method, China

11.2 Author summary

An estimated 30-100 million people are infected with the parasitic worm *Strongyloides stercoralis*, the causative agent of strongyloidiasis, and yet this is a neglected tropical disease. The diagnosis of this parasite requires specialized techniques (e.g. Baermann and Koga agar plate method), but these are rarely employed in epidemiologic studies. We assessed the occurrence of *S. stercoralis* in a rural part of southern Yunnan province, China, and compared different diagnostic methods. At least two stool samples were obtained from 180 randomly selected individuals, and examined with four diagnostic approaches, including the Koga agar plate and the Baermann technique. Twenty-one individuals were infected with *S. stercoralis* (prevalence: 11.7%). Males were more often infected than females (18.3% *versus* 6.1%, $P = 0.011$). Infections were absent in children below the age of 15 years. The Baermann technique showed a higher sensitivity than the Koga agar plate method, and the examination of multiple stool samples improved the diagnostic performances of both methods. The use of a mathematical model suggested a 'true' *S. stercoralis* prevalence of 16.3%. There is a need to further study the epidemiology of strongyloidiasis in other parts of China, and control measures are required in settings with high prevalences as observed in this area.

11.3 Introduction

Soil-transmitted helminthiases are caused by infections with intestinal nematodes, of which *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are the most widespread species [1-3]. Collectively, these soil-transmitted helminths affect over 1 billion people and cause a huge public-health burden; yet, soil-transmitted helminthiases are so-called neglected tropical diseases [4]. *Strongyloides stercoralis* is another and even more neglected soil-transmitted helminth, although an estimated 30-100 million people are infected worldwide [2]. An infection with *S. stercoralis* occurs transcutaneously and can be perpetuated over long periods by autoinfection [5, 6]. Clinical signs of *S. stercoralis*-infected immunocompetent people can be inconspicuous or even absent, but hyperinfection involving the gastrointestinal and pulmonary system is possible. Potentially fatal disseminated infections are seen in immunocompromised individuals, for example, as a result of immunosuppressive drugs or following human T-cell lymphotropic virus type 1 (HTLV-1) infection [6-8].

S. stercoralis is endemic in tropical and temperate zones but accurate information on the geographic distribution and the global burden of strongyloidiasis is lacking. An important underlying reason is that one of the most widely used diagnostic approaches in helminth epidemiology, i.e., the Kato-Katz method [9], fails to detect *S. stercoralis*. Moreover, microscopic examination of direct fecal smears, often used in endemic settings, has a low sensitivity [10, 11]. More sensitive diagnostic approaches for detection of *S. stercoralis* larvae include the Koga agar plate method [12] and the Baermann technique [13]. Their sensitivity can be further increased by examining multiple stool samples [14].

In East Asia and Thailand in particular, the epidemiology of *S. stercoralis* has been studied in some detail. In different investigations carried out among schoolchildren and adults in northern and central Thailand, prevalences ranging between 2.3% and 28.9% were found [15-19]. *S. stercoralis* has also been investigated in other Asian countries, including Japan [20], but there is a paucity of epidemiologic data and comparison of different diagnostic methods from China. This can be illustrated by consulting the *PubMed* database (http://www.pubmed.gov) where the following search strategy "strongyloides OR strongyloidiasis AND China" resulted in only 6 hits; 3 case reports, 1 study on animal strongyloidiasis, 1 global review, and 1 old publication that looked at single and multiple species parasitic infections among 15,952 Chinese using direct-smear examinations [21] (accessed on 29 June 2007).

Here, we report findings from a cross-sectional parasitologic and questionnaire survey carried out in a random population sample in a rural setting of southern Yunnan province, China. We investigated the occurrence of *S. stercoralis* by screening multiple stool samples from the same individuals and comparatively assessed the performance of different diagnostic methods.

11.4 Materials and methods

11.4.1 Study area and population

The study was carried out in Nongyang village, located in Menghai county, Xishuangbanna prefecture, Yunnan province, China (21.81° N latitude and 100.35° E longitude). The village was selected because (i) the hookworm prevalence in this area is known to be high (used as a proxy for the likely occurrence of *S. stercoralis*, as both species have the same way of transmission), and (ii) it is readily accessible by project car to assure a rapid transfer of stool samples to the nearby laboratory. Details of the study village and the population sample have been presented elsewhere [22]. In brief, the village is inhabited by members of the Bulang ethnic group, and is situated 20 km southwest of the town of Menghai in a hilly area at an elevation of 1350 m above sea level. The economy of the village is governed by the surrounding tea and sugar cane plantations, other sources of income than farming are not available. Pigs and poultry are the most common domestic animals, others include dogs and buffaloes. Whilst all houses have untreated tap water originating from a nearby river, there are no household-based sanitation facilities. A single community latrine serves the entire population, but it is not consistently used.

11.4.2 Consent, field and laboratory procedures

The village authorities were informed about the study, and a copy of the village family registry, containing basic demographic information, was obtained. According to the village family registry, there were some 150 households. Families with odd registration numbers (n = 78) were contacted in batches of 20-30 families per week, and all members were invited to participate in the survey. The aim and procedures of the study were explained, and an informed consent sheet was signed by the head of the household or a designated literate substitute. Pre-tested individual and household questionnaires were administered to obtain demographic (age, sex, education attainment), behavioral (wearing shoes, food consumption, personal hygiene, health care seeking) and occupational data, as well as information about the

living conditions (household asset ownership, house type, sanitation infrastructure, domestic animals). Next, pre-labeled plastic containers for stool sample collection were handed out to all participants and their ability to recognize their names was checked. Each morning, filled containers were collected and replaced by empty ones for stool collection on the following day. This procedure was repeated with the goal to obtain 3 stool samples from each individual.

The stool samples were stored at ambient temperature and transferred to the laboratory within 2 hours post-collection. They were processed by the Kato-Katz technique [9], the Baermann method [13] and the Koga agar plate procedure [12]. In addition, one sub-sample per study participant was stored in sodium acetate-acetic acid-formaline (SAF) solution, forwarded to a reference laboratory in Switzerland, and processed there by an ether-concentration method for the examination of helminth eggs and intestinal protozoa [23]. All tests were performed according to standard operating procedures and carried out or initiated within 12 hours after sample collection.

Specifically, a single Kato-Katz thick smear was prepared from each stool sample and examined within 1 hour of preparation. Helminth eggs were counted separately to obtain parasite-specific infection intensity estimates. For the Baermann test, an apricot-sized stool sample was placed on a gauze-lined mesh in a glass funnel equipped with a rubber tube and a clamp, covered with deionised water and illuminated from below with a bulb. After 2 hours, the lowest 50 ml of the liquid were drained, centrifuged and the sediment examined under a microscope for *S. stercoralis* larvae (L1-stage). The Koga agar plates were freshly prepared once per week and kept at 4°C in humid conditions pending utilization. A hazelnut-sized stool sample was placed in the middle of the plate and the covered plates were incubated in a humid chamber for 2 days at 28°C. All plates were rinsed with 12 ml SAF solution, the eluent centrifuged and the sediment examined under a microscope. Recovered larvae were differentiated to distinguish *S. stercoralis* L3 larvae from hookworm larvae. Samples were considered positive if larval or adult *S. stercoralis* were observed.

11.4.3 Statistical analyses

Questionnaire data were entered in EpiData version 3.0 (EpiData Association; Odense, Denmark) and statistical analyses were carried out in STATA version 9.2 (StataCorp.; College Station, USA). Prevalence estimates for *S. stercoralis* according to the Koga agar plate and the Baermann methods were calculated by means of a mathematical model presented and used elsewhere [24, 25]. Based on the relative frequency of single and repeated

positive test results among the multiple stool samples submitted by the participants, the model extrapolates a 'true' prevalence and calculates additional test characteristics for a given method.

11.4.4 Anthelminthic treatment and ethical considerations

At completion of the study, free treatment with compound mebendazole (i.e., mebendazole 100 mg/tablet plus levamisole hydrochloride 25 mg/tablet; 2 tablets per day for 3 consecutive days) was offered to all inhabitants of the village by staff of the local parasite control station.

The institutional review boards of the National Institute for Parasitic Diseases (Shanghai, China) and the Swiss Tropical Institute (Basel, Switzerland) approved the study. As mentioned before, written informed consent was sought from household heads or appropriate literate substitutes.

11.5 Results

11.5.1 Population sample and study cohort

In total, 283 individuals from 71 families participated in the survey (average family size: 4.0 people; range: 1-8). At least 1 stool sample of sufficient quantity to perform the various diagnostic tests was available from 234 individuals (82.7%). Two or 3 samples were submitted by 180 individuals (63.6%) and subsequent analyses were performed on this cohort. There were 98 females (54.4%) and the age of the participants ranged from 4 to 84 years. Among those aged 15 years and above, 92.0% were farmers, the others were students. The illiteracy rate in the same age group was 67.2%. The majority of those aged 14 years and below attended school (58.5%), whereas the remaining individuals were either pre-school children (26.8%) or had never attended school.

11.5.2 Occurrence of S. stercoralis

Fourteen different parasite species were identified, 7 helminths and 7 intestinal protozoa. Very high prevalences of *A. lumbricoides* (93.3%), *T. trichiura* (88.9%) and hookworms (87.8%) were found. Here, we focus on the *S. stercoralis* results. Stool examination utilizing the Koga agar plate and the Baermann technique resulted in the identification of 19 and 21 *S. stercoralis* infections, respectively. As summarized in Table 11.1, all *S. stercoralis* infections detected by the Koga agar plate method were also diagnosed by the Baermann

technique, whereas 2 infections were identified by the latter method only. Thus, the observed infection prevalence of *S. stercoralis*, according to Baermann was 11.7%. The Kato-Katz method and the ether-concentration technique on SAF-conserved stool specimens failed to identify even a single infection with *S. stercoralis*.

Table 11.1 Comparison of results obtained by the Koga agar plate and the Baermann methods for the diagnosis of *S. stercoralis* among 180 individuals with at least 2 stool samples examined in Nongyang village, Yunnan province, China

	Baermann test		Total
	Positive	Negative	
Koga agar plate test positive	19	0	19
Koga agar plate test negative	2	159	161
Total	21[a]	159	180

[a] The sensitivity of the Koga agar plate method was 90.5% and that of the Baermann method was 100% if the combined results from both tests are considered as diagnostic 'gold' standard.

Table 11.2 shows that the prevalence of *S. stercoralis* was significantly higher among males than females (18.3% *versus* 6.1%, $\chi^2 = 6.42$, degrees of freedom (df) = 1, $P = 0.011$) and increased with age, albeit not significantly ($\chi^2 = 8.70$, df = 4, $P = 0.069$). No infections were found among participants <15 years, whereas the highest prevalence was recorded in those aged 15-24 years (19.6%). *S. stercoralis* infections were not found among students of any age. No additional risk factors for a *S. stercoralis* infection could be identified. Neither protective measures against infection, such as wearing shoes (odds ratio (OR) = 0.64, $P = 0.516$), nor hygiene behavior, e.g., hand washing before eating (OR = 1.03, $P = 0.963$) or after defecation (OR = 1.23, $P = 0.671$), willingness to see a doctor in case of illness (OR = 2.91, $P = 0.310$) or presence of domestic animals (e.g., dogs; OR = 1.88, $P = 0.267$) were associated with infection status.

Table 11.2 Number and percentage of study participants infected with *S. stercoralis* as determined by the combined Koga agar plate and Baermann techniques, stratified by sex, age group and occupation among 180 individuals from Nongyang village in Yunnan province, China

Characteristics	No. of individuals examined	No. of individuals positive for *S. stercoralis*	Percent positive	χ^2	*P*-value
All individuals	180	21	11.7		
Sex					
Female	98	6	6.1		
Male	82	15	18.3	6.42	0.011
Age group (years)					
≤9	17	0	0		
10-14	20	0	0		
15-24	46	9	19.6		
25-39	53	5	9.4		
≥40	44	7	17.5	8.70	0.069
Occupation[a]					
Pre-school, student	40	0	0		
Farmer	138	19	13.8	6.17	0.013

[a] Only those individuals with known occupation were included (n = 178).

11.5.3 Performance of different diagnostic methods

Indicators of the diagnostic performance of the Koga agar plate and the Baermann methods, in relation to different sampling efforts, are presented in Table 11.3. The examination of 3 stool samples, rather than a single one, resulted in a significant increase in the number of infections detected by either method. The observed *S. stercoralis* prevalence increased from 7.3% to 11.7% when using the Koga agar plate method (an increase of 62%), and from 7.0% to 14.0% in the case of the Baermann method (an increase of 100%). Whilst using Koga agar plates, larvae were detected with equal frequencies in only 1, 2 or all 3 stool samples from infected individuals, the Baermann method often failed to detect larvae in multiple samples from the same person. Using the results of the Koga agar plate method and a mathematical model developed by Marti and Koella [24], we estimated a 'true' *S. stercoralis* prevalence of 12.3%. The corresponding value for the Baermann technique was 16.3%. The probability of correctly identifying infected individuals by analyzing single stool samples was estimated at 0.63 and 0.48 for the Koga agar plate and the Baermann technique, respectively.

Table 11.3 Identification of *S. stercoralis* larvae by the Koga agar plate and the Baermann methods in 3 different stool samples obtained from inhabitants of Nongyang village in Yunnan province, China, and 'true' prevalence and test characteristics according to a model developed by Marti and Koella (1993) [24]

	Koga agar plate method		Baermann method	
Sampling effort	Number	%	Number	%
3 stool samples analyzed	179	100	129	100
Cumulative result after analysis of				
1st stool sample	13	7.3	9	7.0
2nd stool sample	20	11.2	14	10.9
3rd stool sample	21	11.7	18	14.0
Larvae recovered from				
1 stool sample	7	3.9	9	7.0
2 stool samples	7	3.9	6	4.7
3 stool samples	7	3.9	3	2.3
1, 2 or 3 stool samples	21	11.7	18	14.0
Estimated prevalence (SD)		12.3 (±5.1)		16.3 (±7.6)
Sensitivity of method (3 samples)		95.1		85.6
Sensitivity of individual test (SD)		63.4 (±13.8)		47.5 (±17.1)

SD: standard deviation

Table 11.4 shows the effect of the sampling effort for multiple stool sample collection on the observed prevalence and the influence of the available stool quantity on the completeness of the diagnostic results. Three Koga agar plate tests could be performed for 70.5% of the 254 participants who submitted at least 1 sufficiently-large stool sample. The higher requirements of the Baermann method regarding the available stool quantity are reflected in the lower number of tests. Only 236 participants had at least one Baermann result, whereas 129 (54.7%) submitted 3 large enough stool samples. One *S. stercoralis* infection was identified by the Koga agar plate method among those participants who submitted stool samples of insufficient quantity to concurrently perform the Baermann test. Combined, the Koga agar plate and the Baermann technique identified 30 *S. stercoralis* infections among 254 individuals who submitted at least 1 stool sample of sufficient quantity to perform at least the Koga agar plate test, resulting in an observed prevalence of 11.8%.

Table 11.4 Effect of sampling efforts for stool collection and evaluation with the Koga agar plate and Baermann technique on the observed prevalence, total number of identified infections and the completeness of datasets

Number of stool samples from partici-pants	Koga agar plate method							Baermann method							*S. stercoralis* from 1-3 stool samples; Koga agar plate and/or Baermann method					
	Number of partici-pants	1st sample positive		1st and/or 2nd sample positive		1st and/or 2nd and/or 3rd sample positive		Number of partici-pants	1st sample positive		1st and/or 2nd sample positive		1st and/or 2nd and/or 3rd sample positive		Koga		Baermann		Koga & Baermann	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
≥1	254	19	7.5	n.a.		n.a.		236	19	8.1	n.a.		n.a.		27[c]	10.6	29	12.3	30	11.8
≥2	215	13[a]	6.0	20	9.3	n.a.		180	11[a]	6.1	17	9.4	n.a.							
3	179	13[a]	7.3	20[b]	11.2	21	11.7	129	9[a]	7.0	14[b]	10.9	18	14.0						

[a] Results from 1st sample only
[b] Results from 1st and 2nd sample only
[c] 26 cases jointly diagnosed by the Koga agar plate and the Baermann methods, 1 additional case for which no Baermann test could be performed
n.a.: not applicable

11.6 Discussion

There is a paucity of parasitologic and epidemiologic investigations pertaining to *S. stercoralis* in China, and to our knowledge the performance of different diagnostic approaches has never been assessed in this setting. We carried out an in-depth study in a random population sample from a small village in Yunnan province in the south-western part of China. The collection of multiple stool samples and their screening by the Koga agar plate and the Baermann techniques revealed a prevalence of *S. stercoralis* of 11.7%.

It is conceivable that the observed prevalence still underestimates the 'true' prevalence, which is justified on the following grounds. First, in the absence of a diagnostic 'gold' standard, it is not possible to determine how often larvae failed to emigrate from the stool sample, or actually resided on the surface of the agar plate, but were not recovered. With regard to the Baermann technique, it is possible that some larvae had not yet reached the water, or settled to the ground of the funnel when the water was drained after 2 hours of exposure to light. Second, a recent study carried out in rural Malawi showed that a delay of 3 hours or more between evacuation of stool specimens by humans and processing/examining of stool samples in the laboratory resulted in a considerably decreased sensitivity of hookworm diagnosis [26]. Hence, there is concern that delays in stool processing might also negatively influence the sensitivity of diagnosing other helminth infections, including *S. stercoralis*. Future studies should investigate the effect of time from stool evacuation to laboratory examination with an emphasis on *S. stercoralis*. Third, a mathematical model [24] predicted a considerably higher prevalence of *S. stercoralis* when compared to the results of 3 stool specimens subjected to either the Koga agar plate or the Baermann technique. The application of other diagnostic methods, such as the charcoal coproculture method, which includes a culture step before harvesting the larvae by the Baermann method, and serology, might detect additional infections. Yet, based on our previous experience, we are confident that the approach taken in the current study (multiple stool samples and different diagnostic methods) detected *S. stercoralis* infections with a high sensitivity. Nonetheless, serological methods suitable to also identify very light infections should be used in future studies to further investigate the conspicuous absence of infections among children.

On the other hand, the collection of stool samples over several days under limited supervision by our research team bears the risk of mixing up collection containers at the household level. This would result in the attribution of samples from one infected person to different household members who might not be infected, thus inflating the prevalence. We are

confident that this issue did not distort our data, as we provided detailed explanations to all study participants about the importance of stool collection using the designated containers, and checked the ability of at least one household member to recognize each name on the pre-lab containers. Moreover, the age and sex distribution of *S. stercoralis* infections matched the previously presented epidemiologic patterns from neighboring countries. The 21 infections diagnosed by the Baermann approach originated from 18 families, suggesting that mis-attribution was certainly not a major issue. We also assume that the participation of only 63.6% of the eligible villagers did not affect the representativeness of the sample since the age and sex distribution of these 180 individuals was similar to the remaining 103 people who failed to provide at least 2 stool samples of sufficient quantity.

Concerning the recovery of larvae from the agar plates, an attempt was made to first visually inspect the plate for larval tracks and characteristic signs of fungal and bacterial growth, but the high prevalence of hookworm larvae necessitated the recovery of the actual larvae for microscopic examination. In some cases signs of larval activity were noted, but no larvae could be recovered. Contrarily, it was shown that larvae can be present even if no signs of their activity can be detected on the surface of the agar plate [12].

We are not aware of previous community-based studies focusing on *S. stercoralis* in Yunnan province. The overall prevalence of *S. stercoralis* (11.7%) is similar to reports from northern Thailand [17]. Interestingly, southern Yunnan shares some eco-epidemiologic characteristics with northern Thailand, such as the climate, land use patterns and ethnic background. Moreover, in both settings, the prevalence of infection was significantly higher in males than in females [15, 17], and increased with age, with the peak prevalence observed in adolescents and young adults [18]. Similar sex and age patterns were also reported from Laos [27]. However, in Laos and Thailand, infections were also found among children, whereas in the current study, infections were confined to individuals aged 15 years and above. These findings might point to age- and gender-specific occupational risk factors, e.g., different behavioral patterns related to agricultural activities. The absence of infections among children suggests that the main transmission sites are outside the core village, despite the precarious sanitary conditions with 86.5% of the participants reporting not using the single community latrine available in the entire village. Possibly as a result of the rather uniform educational, occupational and behavioral population characteristics, we were unable to identify additional risk factors for infection.

It is commonly assumed that even if multiple stool samples are available, no single diagnostic technique can detect all *S. stercoralis* infections. Different methods are therefore

employed for the parasitological diagnosis of this helminth but they are often poorly standardized and their performance has rarely been assessed comparatively. In one of the few available studies that compared the diagnostic performance between the Koga agar plate and the Baermann method, the former technique was superior to the Baermann technique [28]. In the present study, however, the Baermann technique identified 'all' infections, whereas the Koga agar plate method failed to do so in 3 cases when considering all individuals who provided at least 1 stool sample of sufficient quantity (Table 11.4). Even taking into account the somewhat lower sensitivity of the Koga agar plate method, this technique still has advantages in field-based epidemiologic surveys. First, it allows the analysis of small stool samples, thereby reducing the number of participants who have to be excluded from the analysis due to insufficient amounts of stool, as was the case in the current study (note the total numbers of Koga agar plate and Baermann technique test results in Table 11.4). Second, the Koga agar plate technique also detects hookworm infections, thus allowing for concurrent diagnosis of both parasites [29]. Previous studies have shown that formaline-ether concentration methods were able to detect *S. stercoralis* infections, but compared to the Baermann and Koga agar plate methods, their sensitivity was considerably lower [10, 16, 30]. The low sensitivity of direct fecal smears and the Kato-Katz method for diagnosis of *S. stercoralis* is also well known [11].

Over the past decades, profound demographic, ecologic and socio-economic changes have occurred across China [31, 32], and the health system underwent significant reforms [33]. These changes also resulted in an increased availability and use of sophisticated medical techniques, including immunomodulatory drugs and organ transplantation. Consequently, it must be assumed that the immunocompromised population is expanding. Previous research has indicated that this population group is at high risk of severe disease when concurrently infected with *S. stercoralis*. Nevertheless, the obvious importance of *S. stercoralis* for public-health has yet to prompt new research into the epidemiology and control of this neglected helminth infection in China and elsewhere. In this connection, the importance of differential diagnosis of soil-transmitted helminth infections must be emphasized, particularly in view of the large-scale administration of albendazole and/or mebendazole that usually show good efficacy against *A. lumbricoides* and hookworms (only moderate efficacy against *T. trichiura*), but commonly fail to clear *S. stercoralis* [1]. We have launched additional studies with the objective of enhancing our understanding of the epidemiologic situation of *S. stercoralis* in adjacent parts of Yunnan province with different environmental, socio-economic and ethnic characteristics, and will also investigate current and future treatment options. Finally, we

encourage other groups who focus their research on helminths, not to neglect *S. stercoralis* any longer.

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12. Tribendimidine and albendazole for treating soil-transmitted helminths, *Strongyloides stercoralis* and *Taenia* spp.: open label randomized trial

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12.1 Abstract

Background Tribendimidine is an anthelminthic drug with a broad spectrum of activity, and has been approved by Chinese authorities in 2004. Efficacy against soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) has been established and new laboratory investigations point to activity against cestodes and *Strongyloides ratti*.

Methodology/Principal Findings In an open-label randomized trial, the safety and efficacy of single-dose oral tribendimidine (200 mg for 5 to 14-year-old children, and 400 mg for individuals ≥15 years) against soil-transmitted helminths, *Strongyloides stercoralis*, and *Taenia* spp. was compared to single-dose oral albendazole in a setting of extensive intestinal polyparasitism in Yunnan province, People's Republic of China. Among 57 individuals who received tribendimidine, the prevalence of *S. stercoralis* was reduced from 19.3% to 8.8% (observed cure rate 54.5%, $P = 0.107$), and that of *Taenia* spp. from 26.3% to 8.8% (observed cure rate 66.7%, $P = 0.014$). Similar prevalence reductions were noted among the 66 albendazole recipients. Taking into account "new" infections discovered at treatment evaluation, which were most likely missed pre-treatment due to the lack of sensitivity of our diagnostic approach, the difference between the drug-specific net cure rates of *Taenia* spp. infections was highly significant in favor of tribendimidine ($P = 0.001$). Both single-dose oral albendazole and tribendimidine were highly efficacious against *A. lumbricoides* and, moderately, against hookworm. The efficacy against *T. trichiura* was low.

Conclusions/Significance Our results suggest that single-dose oral tribendimidine is safe in settings with extensive polyparasitism and its efficacy against *A. lumbricoides* and hookworm is confirmed. The promising results obtained with tribendimidine against *S. stercoralis*, and *Taenia* spp. warrant further investigations. In a next step, multiple-dose schedules should be evaluated.

Keywords tribendimidine; albendazole; soil-transmitted helminths; *Strongyloides stercoralis*; *Taenia*; treatment; diagnosis; People's Republic of China

12.2 Author Summary

A large proportion of the human population is infected with intestinal worms and many people in the developing world harbor several kinds of worms concurrently. There are only a few drugs available against these parasites, and their efficacy varies depending on the species. We compared the efficacy of single-dose oral tribendimidine, a new broad-spectrum worm drug from China, with the standard drug albendazole for the treatment of hookworm, large roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and, for the first time, *Strongyloides stercoralis* and tapeworm (*Taenia* spp.). Our single-blind randomized trial was conducted in a setting of extensive intestinal multiparasitism in Yunnan province, south-west China. Among 57 individuals who received tribendimidine, the prevalence of *S. stercoralis* was reduced from 19.3% to 8.8%, and that of *Taenia* spp. from 26.3% to 8.8%. Similar prevalence reductions were noted among the 66 albendazole recipients. Taking into account additional infections only discovered at treatment evaluation, the difference between the drug-specific *Taenia* spp. net cure rates was highly significant in favor of tribendimidine. Both drugs showed high efficacy against *A. lumbricoides* and a moderate effect against hookworm. In view of our promising results, multiple-dose schedules against *S. stercoralis* and *Taenia* spp. should be evaluated in a next step.

12.3 Introduction

There is growing awareness of the intolerable burden due to the so-called neglected tropical diseases [1]. Hence, new initiatives are underway for their control [2]. For helminth infections, the mainstay of control in high-burden areas rests on regular administration of anthelminthic drugs [1-5]. However, only a handful of drugs are available that have been developed many years ago [6], and there is considerable concern that resistance might develop, e.g., following exposure to sub-curative doses. Reduced efficacy of common anthelminthic drugs is a major problem in veterinary medicine but for humans, it is of no clinical relevance thus far [7]. In a world where intestinal polyparasitism is common, yet neglected [8-12], and widely used treatment regimens are only effective against a limited number of species [6], some helminths might increase in relative frequency, e.g., *Strongyloides stercoralis* and *Taenia* spp.

High prevalences of soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and, consequently, a high prevalence of intestinal multiparasitism, have recently been described from Nongyang, a settlement in Manguo village, located in southwest Yunnan province, People's Republic of China [13]. Whilst *S. stercoralis* is endemic in the People's Republic of China [14], the local epidemiology of this parasite is not well understood. A prevalence of approximately 15% was found in Nongyang [15]. Taeniasis and infections with the larval stage of *Taenia solium*, i.e., cysticercosis, have been documented throughout the People's Republic of China. Most infections occur in counties inhabited by non-Han nationalities whose traditional diets include the consumption of raw or undercooked meat [14,16]. However, there is a paucity of epidemiologic data for taeniasis from the People's Republic of China, at least in the English literature [17]. In a recent cross-sectional survey, we found an egg-prevalence of *Taenia* spp. of 3.5% among 3220 individuals in Eryuan county, northwest Yunnan province [18]. In Nongyang, the prevalence of *Taenia* spp. was 5.1% [13].

The benzimidazoles, i.e., albendazole and mebendazole, are the most widely used drugs for the control of soil-transmitted helminthiasis [1,19-21]. Both drugs have some effect against *S. stercoralis* and *Taenia* spp., but triple doses are recommended to achieve high cure rates [6,22]. The drug of choice for treating *S. stercoralis* is ivermectin [23]. Praziquantel and niclosamide are the recommended drugs against *Taenia* spp. [24-27]. Large-scale administration of ivermectin is the cornerstone of control programs targeting filarial infections, most notably onchocerciasis [28]. However, *Onchocerca volvulus* is not endemic in the People's Republic of China. Additionally, ivermectin is highly efficacious against

A. lumbricoides, shows some activity against *T. trichiura*, but fails to cure hookworm infections. Since hookworm infections are common in the People's Republic of China, ivermectin is not used in soil-transmitted helminth control. These issues might explain why ivermectin is registered for human use in the country, but not readily available; ivermectin, however, is produced at large scale for veterinary medicine, the bulk of which is exported.

Tribendimidine is an anthelminthic drug that has been registered in the People's Republic of China for use in humans [6,29]. Tribendimidine is a symmetrical diamidine derivative of amidantel [30], its CAS registration number is 115103-15-6. Used at the current standard dose of 200 mg for children aged 5-14 years, and 400 mg for individuals aged ≥15 years, tribendimidine is save and efficacious against *A. lumbricoides*, hookworm, and *Enterobius vermicularis* [29,31,32]. It also shows some activity against *T. trichiura* [29,31,32], cestodes [29], and some trematodes [30]. New research revealed in vitro and in vivo activity of tribendimidine against *Strongyloides ratti* [33].

The objective of this study was to assess the safety and efficacy of single-dose oral tribendimidine for treating intestinal helminth infections in a rural setting where polyparasitism is common. Here, we focus on the effects of tribendimidine on *S. stercoralis* and *Taenia* spp., and summarize the results pertaining to the common soil-transmitted helminths. Comparison is made with single-dose oral albendazole as half of the participants were administered either drug.

12.4 Materials and methods

12.4.1 Study site and population

The study was carried out in Nanweng, a village in Menghai county, Xishuangbanna prefecture, Yunnan province, People's Republic of China, from May to July 2007. Nanweng is situated on the slope of a mountain, 1650 m above sea level at 21.77 N latitude and 100.40 E longitude. The village is exclusively inhabited by the Bulang ethnic group; its economic basis is provided by the surrounding tea plantations and the more distant, partially irrigated rice and other crop fields. Untreated tap water is delivered to every house but no sanitation facilities are available in the entire village.

12.4.2 Field and laboratory procedures

The leader of the Menghai-based county station for the control of parasitic diseases briefed the village authorities about the study. Village leaders then informed the residents who were

all invited to participate. Over a 3-week period, 20-30 families were enrolled weekly and household as well as individual questionnaires that have been used before [15,18] were administered. Children below the age of 15 years were assisted by their parents or legal guardians to answer the questions. Infants younger than 2 years were excluded from the study. Participants were asked to provide a large stool sample in pre-labeled collection containers. Filled containers were collected daily and exchanged by empty ones with the goal to obtain 3 stool samples per participant. The evaluation of the treatment efficacy commenced 2 weeks post-treatment and followed the same field procedures. Stool samples were collected over a 2-week period, again aiming to obtain 3 samples per study participant.

Stool samples were collected in the village between 7 and 9 a.m., transferred to the laboratory in Menghai city, and processed within a maximum of 6 hours of receipt. First, ~10 g of stool were placed on a gauze which was embedded on a wire mesh in a glass funnel, equipped with a sealable rubber tube, the so-called Baermann device [34]. The funnel was then filled with de-ionized water and illuminated from below with an incandescent bulb. Second, for the Koga agar plate test [35], ~2 g of stool were placed in the centre of a 9 cm Petri dish with freshly prepared nutrient agar. Third, a single 42 mg Kato-Katz thick smear [36] was prepared on a microscope slide and helminth eggs were enumerated after a clearing time of 30-60 min.

The lowest 45 ml of the liquid in the Baermann funnel were drained after 2 h, centrifuged and the sediment was examined for *S. stercoralis* larvae at low magnification (40 X). Koga agar plates were inspected for helminth larvae at low magnification after a 2-day incubation period at 28°C in a humid chamber. Subsequently, all plates were rinsed with 12 ml sodium acetate–acetic acid–formaline (SAF) solution [37], gently scraped and the eluent was centrifuged. The sediment was examined for helminth larvae, i.e. *S. stercoralis* and hookworms. Samples were considered positive if larvae were detected at any stage. Helminth eggs in the Baermann and Koga sediment were also noted.

12.4.3 Drug administration

Study participants who had submitted at least 1 stool sample were listed according to their identification number, and randomly assigned to either albendazole purchased from Shanxi Hanwang Medicine Co. Ltd. (Han Zhong, People's Republic of China), or tribendimidine obtained from Shandong Xinhua Pharmaceutical Co. Ltd. (Zibo, People's Republic of China). Drugs were administered as single 200 mg oral dose for children aged 5-14 years, and 400 mg

for individuals ≥15 years of age. Our study was an open-label trial, i.e., only participants did not know which drug they received. Participants were asked to avoid alcohol consumption on the day of treatment. After dinner time, teams of fieldworkers visited the village and asked each participant at home about signs of acute or chronic illness, and alcohol consumption. Women aged ≥14 years were asked for pregnancy. Drugs in pre-labeled envelopes were handed out together with fresh bottled water to healthy, non-drunk and non-pregnant participants, and drug intake was observed. Those treated were asked to report any potential drug-related signs or symptoms and sleeping troubles to the accompanying medical doctor.

12.4.4 Ethical considerations and end-of-study treatment

The study was approved by the Institutional Review Board of the Swiss Tropical Institute, Basel, as well as the Ethics Committee of the University and the State of Basel (EKBB), Switzerland (reference no. 149/07) and the Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, People's Republic of China). The study procedures, potential risks, and benefits were explained to the village leaders and after their consent to perform the study, field workers visited the homes of the selected families where detailed information was provided to all potential participants and questions were answered. Emphasis was placed on voluntary participation and the option to quit the study at any moment without further obligation. Written confirmation that full information had been provided and individual participation was voluntary (informed consent) was obtained from the head of each participating household or a literary substitute (adult child or relative), and this procedure was approved by the above-mentioned ethical committees.

A single 200 mg (for children aged 5-14 years), or 400 mg (individuals aged ≥15 years) oral dose of albendazole was offered to those participants who were not eligible for randomization because they had failed to provide any stool sample. The assessment of their health status and the treatment procedures followed the same protocol as for study participants, but the treatment outcome was not assessed.

Locally-used remedies for *Taenia* spp. infection and ivermectin for treating *S. stercoralis* at the standard dose (200 µg/kg) were provided at the end-of-study follow-up. Finally, albendazole was provided to the village authorities for later distribution to untreated inhabitants and participants who still harbored active infections.

12.4.5 Data management and statistical analysis

The questionnaire data were double-entered and cross-checked in EpiData version 3.1 (EpiData Association; Odense, Denmark). The laboratory data were examined for internal consistency, and merged with the questionnaire data. Statistical analysis was done with STATA version 9.2 (StataCorp; College Station, USA). Our final study cohort consisted of individuals aged ≥5 years who had submitted at least 2 stool samples at baseline, did not suffer from any recognized chronic or acute illness, were not pregnant, had not drunk alcohol on the day of drug administration, had taken the randomly assigned drug, and had again 2 or 3 stool samples examined at the end-of-study survey. Multiple stool readings at baseline and follow-up were required in order to boost diagnostic sensitivity [13,15].

The infection status was determined based on the pooled results from the different diagnostic methods (soil-transmitted helminths and *Taenia* spp.: all tests; *S. stercoralis*: Baermann plus Koga agar plate). Pearson's χ^2-test and Fisher's exact test, as appropriate, were used to assess the association between infection and demographic variables. Treatment outcomes by drug and the differences between albendazole and tribendimidine were explored by calculating drug-specific prevalence reductions and analyzing the difference between the observed cure rates (2-sample test of proportions).

12.5 Results

12.5.1 Participation

We counted 294 family members, aged 3-87 years, in the 81 resident families. Another 60 individuals were recorded in the village registry but they had either left the village, were <2 years, or refused to answer the questionnaire. Figure 12.1 shows that 106 (36%) of the eligible participants provided none (n = 57), or only a single (n = 49) stool sample of sufficient quantity to perform all diagnostic tests. The remaining 188 persons (64%) were 5 to 87-year-old and among them, 17 could not be treated due to pregnancy (n = 8), ill health (n = 5) or other reasons (n = 4). The randomization of the 171 participants with complete parasitological baseline data who were eligible for treatment resulted in equal-sized groups for single-dose oral tribendimidine or albendazole administration, but stool sample submission at baseline and actual treatment rates were somewhat lower among tribendimidine recipients.

Of the 91 participants treated with albendazole, 2-3 sufficiently large stool samples were available from 66 (73%) at the end-of-study follow-up. A similar stool sample submission rate was observed for the 80 tribendimidine recipients (71%). The final cohort consisted of

123 individuals who received either albendazole (n = 66) or tribendimidine (n = 57), and submitted at least 4 (2 pre- plus 2 post-treatment) sufficiently large stool samples to carry out the full range of diagnostic tests. While drop-out rates were similar for males and females (42.2% *versus* 41.5%), full participation ranged from 21% for 10 to 14-year-old to 58% for those aged ≥40 years (data not shown).

12.5.2 Helminth infections at baseline

Considering the joint results of the 2-3 Kato-Katz, Koga agar plate, and Baermann tests, our final cohort showed high prevalences of *T. trichiura* (87.8%), hookworm (74.8%), and *A. lumbricoides* (72.4%). The prevalences of *Taenia* spp. and *S. stercoralis* were 26.0% and 17.9%, respectively.

Table 12.1 shows that *Taenia* spp. infections were significantly more prevalent among males (33.9%) than females (18.0%, $\chi^{2} = 4.01$, degrees of freedom (df) = 1, $P = 0.045$) and increased with age, albeit not significantly ($P = 0.163$). An increase with age was also noted for *S. stercoralis*. No *S. stercoralis* were diagnosed among pre-school children and students as opposed to farmers ($P = 0.121$); *Taenia* spp. was also more common among farmers (27.3%) than those in education (8.3%, $P = 0.292$).

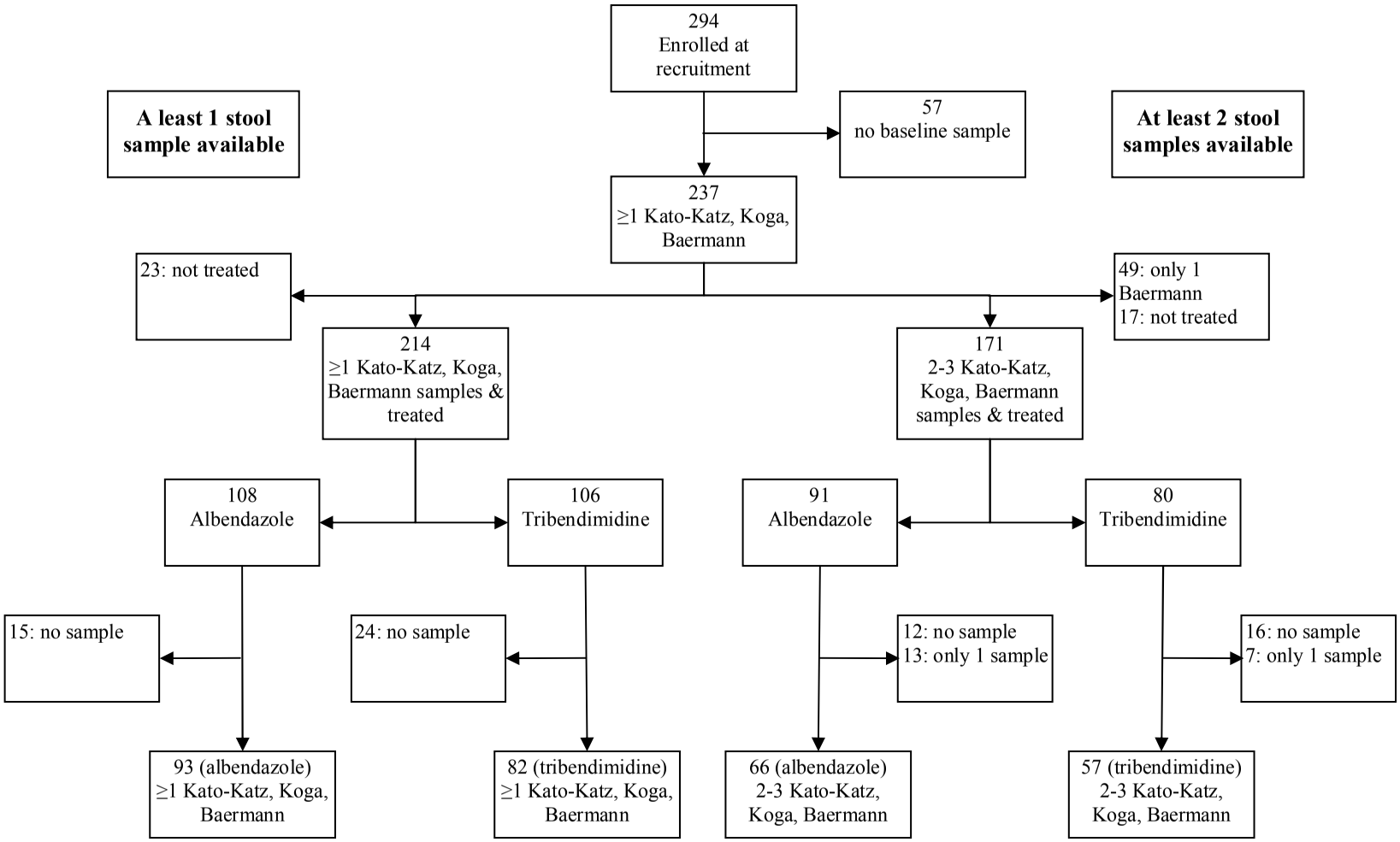

Figure 12.1 Participation and stool sample submission compliance in a community-based open label treatment efficacy study involving repeated stool sample collection and anthelminthic treatment by either albendazole or tribendimidine; Nanweng village, Yunnan province, People's Republic of China.

Table 12.1 Baseline prevalence of *S. stercoralis* and *Taenia* spp. infections among 123 study participants from Nanweng village in Yunnan province, People's Republic of China who had submitted at least 2 stool samples before and after treatment with either albendazole or tribendimidine, stratified by demographic variables.

		Examined	*S. stercoralis*				*Taenia* spp.			
			Positive	% (95% CI)	χ^2	*P*-value	Positive	% (95% CI)	χ^2	*P*-value
Total		123	22	17.9 (11.0, 24.6)	n.a.	n.a.	32	26.0 (18.2, 33.9)	n.a.	n.a.
Sex:	Female	61	10	16.4			11	18.0		
	Male	62	12	19.4	0.18	0.668	21	33.9	4.01	0.045
Age:	5-14 years	15	1	6.7			1	6.7		
	15-24 years	13	1	7.7			2	15.4		
	25-39 years	35	7	20.0			12	34.3		
	40-87 years	60	13	21.7		0.493[c]	17	28.3		0.163[c]
Education[a]:										
	Illiterate	34	9	26.5			9	26.5		
	Primary & middle school	72	12	16.7		0.298[c]	21	29.2		0.821[c]
Occupation[b]:										
	Farmer	110	22	20.0			30	27.3		
	Preschool, student	12	0	0		0.121[c]	1	8.3		0.292[c]

Analysis by Pearson's $\chi 2$-test

[a]: Among those aged ≥18 years; educated group includes 2 participants with higher education (apprenticeship, college)

[b]: 1 participant with other occupation than farmer (civil servant) excluded

[c]: Fisher's exact test

12.5.3 Drug efficacy with an emphasis on Taenia *spp. and* S. stercoralis

As detailed in Table 12.2, single-dose oral albendazole and tribendimidine significantly reduced the prevalence of *A. lumbricoides* (albendazole: from 75.8% to 0; tribendimidine: from 68.4% to 5.3%; both $p<0.001$), and hookworm (albendazole: from 69.7% to 21.2%; tribendimidine: from 80.7% to 38.6%; both $p<0.001$). Whilst the difference between the drug-specific cure rates against *A. lumbricoides* showed borderline significance in favor of albendazole, there was no difference in the efficacy of the two drugs against hookworm. Although neither albendazole nor tribendimidine resulted in a significant reduction in the prevalence of *T. trichiura*, single-dose oral albendazole was significantly more efficacious than tribendimidine in curing *T. trichiura* ($P = 0.014$).

Single-dose oral albendazole and tribendimidine resulted in prevalence reductions of *S. stercoralis* of 6.1% and 10.5%, respectively, which was not statistically significant (both $p>0.05$). The prevalence of *Taenia* spp. was reduced from 25.8% to 10.6% after albendazole administration ($P = 0.024$), whilst among the tribendimidine recipients, the prevalence was lowered from 26.3% to 8.8% ($P = 0.014$). Table 2 also shows that after administration of albendazole, *S. stercoralis* larvae could still be found among 7 of the previously 11 infected individuals (observed cure rate: 36.4%). Among those treated with tribendimidine, 6 out of 11 individuals were larvae-free (observed cure rate: 54.5%; difference: 18.1%, $P = 0.394$). The baseline *Taenia* spp. prevalence of 25.8% and 26.3% among those given albendazole and tribendimidine was reduced by 58.8% and 66.7%, respectively (difference: 7.9%, $P = 0.645$).

Table 12.2 Prevalence and cure rates of *A. lumbricoides*, *T. trichiura*, hookworm, *S. stercoralis*, and *Taenia* spp. among 123 study participants from Nanweng village, Yunnan province, People's Republic of China who submitted at least 2 stool samples before and after treatment with single-dose oral albendazole or tribendimidine.

Drug	Albendazole										Tribendimidine									
Parasite	*A. lumbricoides*		*T. trichiura*		Hookworm		*S. stercoralis*		*Taenia* spp.		*A. lumbricoides*		*T. trichiura*		Hookworm		*S. stercoralis*		*Taenia* spp.	
	n	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence	*n*	Prevalence
Total *n*	66										57									
Baseline: positive	50	75.8	60	90.9	46	69.7	11	16.7	17	25.8	39	68.4	48	84.2	46	80.7	11	19.3	15	26.3
Follow-up: still positive	0	0	53	80.3	14	21.2	7	10.6	7	10.6	3	5.3	48	84.2	22	38.6	5	8.8	5	8.8
Prevalence reduction	75.8 (65.5, 86.1)[a] *P* <0.001		10.6 (-1.2, 22.4)[a] *P* = 0.083		48.5 (33.7, 63.3)[a] *P* <0.001		6.1 (-5.6, 17.8)[a] *P* = 0.307		15.2 (2.3, 28.1)[a] *P* = 0.024		63.1 (49.7, 76.5)[a] *P* <0.001		0 (-13.4, 13.4)[a] *P* = 1.000		42.1 (25.8, 58.4)[a] *P* <0.001		10.5 (-2.1, 23.1)[a] *P* = 0.107		17.5 (3.9, 31.1)[a] *P* = 0.014	
Cured/cure rate	50	100	7	11.7	32	69.6	4	36.4	10	58.8	36	92.3	0	0	24	52.2	6	54.5	10	66.7
Difference drug-specific cure rates	7.7 (-0.7, 16.1)[a] *P* = 0.046		11.7 (3.6, 19.8)[a] *P* = 0.014		17.1 (-2.5, 36.7)[a] *P* = 0.093		18.1 (-22.8, 59.0)[a] *P* = 0.394		7.9 (-25.5, 41.3)[a] *P* = 0.645		7.7 (-0.7, 16.1)[a] *P* = 0.046		11.7 (3.6, 19.8)[a] *P* = 0.014		17.1 (-2.5, 36.7)[a] *P* = 0.093		18.1 (-22.8, 59.0)[a] *P* = 0.394		7.9 (-25.5, 41.3)[a] *P* = 0.645	

[a]: 95% confidence interval

When we took into account *S. stercoralis* and *Taenia* spp. infections that had only been recognized at treatment evaluation (these infections were most likely missed pre-treatment due to lack of diagnostic sensitivity), the efficacy of the drugs was lower (Table 12.3). In both treatment groups, *S. stercoralis* was diagnosed in 2 individuals previously declared uninfected. Hence, the net cure rate was 18.2% for albendazole and 36.4% for tribendimidine recipients (difference: 18.2%, $P = 0.338$). The number of "new" *Taenia* spp. infections in the albendazole group diagnosed during follow-up equaled the number of recoveries ($n = 10$), resulting in a zero overall cure rate. Among tribendimidine recipients, only 2 additional *Taenia* spp. infections were found; the prevalence reduction showed borderline significance (-14.0%, $P = 0.058$). The net cure rate of *Taenia* spp. in the tribendimidine recipients was 53.3%, significantly different from the albendazole group (difference: 53.3%, $P = 0.001$).

Table 12.3 Prevalence and net cure rates taking into account "new" infections discovered at follow-up among previously "negative" participants. Effect of single-dose oral albendazole or tribendimidine for the treatment of *S. stercoralis* and *Taenia* spp. among 123 study participants from Nanweng village, Yunnan province, People's Republic of China who submitted at least 2 stool samples before and after treatment.

	S. stercoralis				***Taenia* spp.**			
	Albendazole		Tribendimidine		Albendazole		Tribendimidine	
	n	Prev./%	*n*	Prev./%	*n*	Prev./%	*n*	Prev./%
Total samples	66	100	57	100	66	100	57	100
Positive before treatment	11	16.7/100	11	19.3/100	17	25.8/100	15	26.3/100
Still positive after treatment	7	10.6/63.6	5	8.8/45.5	7	10.6/41.2	5	8.8/33.3
'New' positive after treatment	2	3.0/18.2	2	3.5/18.2	10	15.2/58.8	2	3.5/13.3
Total positive after treatment	9	13.6/81.8	7	12.3/63.6	17	25.8/100	7	12.3/46.7
Net prevalence reduction	3.1 (-9.1-15.3)[a] $P = 0.619$		7.0 (-6.3-20.3)[a] $P = 0.306$		0.0 (-14.9-14.9)[a] $P = 1.000$		14.0 (-0.3-28.3)[a] $P = 0.058$	
Net cured/net cure rate	2	18.2	4	36.4	0	0	8	53.3
Difference between drug-specific net cure rates	18.2 (-18.2, 54.6)[a] $P = 0.338$				53.3 (28.1, 78.5)[a] $P = 0.001$			

Analysis by two-sample test of proportions
Prev./%.: Prevalence/percentage of initial positives
[a]: 95% confidence interval

12.5.4 Adverse events

No adverse events were mentioned by participants treated with single-dose oral albendazole. In the tribendimidine group, an 87-year-old woman reported mild sleeping disorders, headache, dizziness, and gastrointestinal symptoms, including a single episode of vomiting. This subject had a light infection with *T. trichiura* and hookworm at baseline. Upon treatment

evaluation, this subject did not submit any further stool samples, and hence was excluded from the final cohort.

12.6 Discussion

To our knowledge, this is the first investigation assessing the safety and efficacy of tribendimidine for treating *S. stercoralis* and *Taenia* spp. infections, and the first clinically-monitored use of tribendimidine in a setting with high rates of intestinal multiparasitism. Indeed, infections with one of the three main soil-transmitted helminths were found in 72.4-87.8% of the study participants, and only 4 of the 123 individuals in our final cohort (3.3%) harbored none of these soil-transmitted helminths. The prevalence of *S. stercoralis* and *Taenia* spp. at baseline was 17.9% and 26.0%, respectively. Our study was an open-label trial, comparing single-dose oral albendazole with single-dose oral tribendimidine.

Our final study cohort comprised less than 50% of those initially contacted. Whilst the cohort had a similar sex distribution than the total population of Nanweng village, it was considerably biased toward older age groups. We screened 2-3 stool samples for intestinal helminths and randomly assigned the participants to either albendazole or tribendimidine. Treatment outcome was assessed 2-4 weeks after dosing using multiple stool samples and a diversity of diagnostic approaches. The prevalence of *S. stercoralis* was not significantly reduced by either albendazole or tribendimidine, and no significant drug-specific difference was observed. Among individuals infected with *Taenia* spp. at baseline, the observed cure rates of 58.8% for albendazole and 66.7% for tribendimidine showed statistical significance (both $p<0.05$). During follow-up, however, additional infections were found, mainly *Taenia* spp. among those who had received albendazole, and the difference between the drug-specific overall cure rates was 53.3% ($P = 0.001$). The observed cestocidal effect of tribendimidine, for the time being, should rather be regarded as an indication of a possible activity than as a proof-of-concept. This is due to the obvious diagnostic challenges encountered in field-based clinical studies.

In the current trial, albendazole generally performed slightly better than tribendimidine in curing common soil-transmitted helminth infections. The most notable difference was seen with *T. trichiura*, confirming earlier observations that albendazole is somewhat more efficacious than tribendimidine against this nematode [6,29]. The observed cure rates against hookworm and *T. trichiura* following single-dose oral albendazole were rather low compared to the results of a recent meta-analysis with this and other anthelminthic drug against common

soil-transmitted helminthes [21]. We speculate that this observation is rather reflecting the rigorous diagnostic approach employed than an unusually low susceptibility of hookworm and *T. trichiura* to albendazole. For example, hookworm infections could not only be detected by the widely used Kato-Katz technique, but also by the Koga agar plate method. However, the low cure rates observed in this study should be seen as a warning sign and call for rigorous monitoring of drug efficacy and the potential emergence of drug resistance [38].

The inclusion of only 123 individuals who met our rigorous sample submission requirements into the final study cohort reduced the reported compliance rate but increased the reliability of the results due to the increased overall sensitivity of the employed methods [15]. Indeed, a lower prevalence was found among those 175 participants who had at least 1 stool sample analyzed, but the drug-specific efficacies were similar (data not shown).

The discovery of notable numbers of infections among those who were deemed negative before treatment can be explained by at least 2 mechanisms, or a combination thereof. First, it is conceivable that the baseline evaluation fell within the prepatent period of recent infections. Second, it is well known that parasitological diagnosis of both *S. stercoralis* [39] and *Taenia* spp. [26,40] lacks sensitivity. For *S. stercoralis*, the main remedy for this challenge is testing of multiple stool specimens [23], whilst for *Taenia* spp., sensitive coproantigen enzyme-linked immunosorbent assay (ELISA) tests provide valuable alternatives [24,26,27,40]. The current diagnostic 'gold' standard to confirm treatment success in taeniasis is the recovery of the tapeworm scolex. Alternatively, the absence of proglottids from stools and underwear over a period of 3 months also provides solid proof of cure. However, such extensive observation is usually only feasible in hospital settings. Re-infection after treatment can almost certainly be excluded for *Taenia* spp., and it is rather unlikely for *S. stercoralis*. We speculate that in our study, the limited sensitivity of the diagnostic tools was more significant since the 3 to 5-week period between baseline and follow-up investigation is rather short for any notable numbers of recent infections.

We are confident that our results are valid despite the imperfect sensitivity of the employed diagnostic tests, not least due to our rigorous sampling effort. This assumption is backed by the following observations. For *S. stercoralis*, the numbers of "new" infections at follow-up was similar in both treatment groups (both $n = 2$), thus reducing the observed cure rate but not affecting the overall conclusion that both drugs exhibit some effect at the employed dosage. A mathematical model [41] for the prediction of "true" prevalence further suggested an underestimation of the *S. stercoralis* prevalence by the employed procedures within the range actually observed in the present trial [15]. After a study involving extensive

stool sample collection and analysis by the Baermann technique, Dreyer and co-workers [42] suggested that at least 4 stool samples need to be collected to accurately assess the *S. stercoralis* infection status, and that only those with at least 2 positive test results should be included in clinical drug trials. In our study, we collected 2 to 3 stool specimens and employed 2 diagnostic tools. Thus, 4 to 6 results were available to judge the infection status of the participants both before and after drug administration. Among the 22 *S. stercoralis* positives at baseline, 6, 6, 3, 5, 1 and 1 individuals had 1, 2, 3, 4, 5 and 6 positive test results, respectively. The 10 arguably cured individuals had had 1 (n = 3), 2 (n = 3), 3 (n = 2) and 4 (n = 2) positive baseline test results. Our findings indicate that participants with only 1 or 2 positive tests at baseline were not more likely to be considered cured at treatment evaluation than those with multiple positive tests. However, infections were still found in all participants with 5 or 6 positive tests at baseline. The four individuals who were only found to be infected at treatment evaluation then had 1, 1, 2 and 3 positive test results. Finally, the observed activity of albendazole against *Taenia* spp. among those who were found to be infected at baseline has to be put into perspective with the high number of "newly" detected infections at treatment evaluation. Among tribendimidine recipients, only few additional *Taenia* spp. infections were found, indeed indicating that single-dose oral tribendimidine, but not albendazole, might have some effect against *Taenia* spp

The eggs of *T. saginata*, *T. solium*, and *T. asiatica* cannot be distinguished microscopically [24,26]. Hence, we are not in a position to determine their relative frequency in our study population. However, the reported and observed diets suggest that the locally dominant species is *T. solium* or possibly *T. asiatica* since Bulang favor raw pork over raw beef.

As a next step, the efficacy of multiple-dose oral tribendimidine could be assessed as our results indicate some, albeit currently unsatisfactory effect of this drug against *S. stercoralis* and *Taenia* spp. In future studies with a focus on these 2 parasites rather than the common soil-transmitted helminths, the reference drug should be praziquantel or niclosamide for *Taenia* spp., and ivermectin in the case of *S. stercoralis*. Alternatively, triple-dose albendazole might be used as reference treatment [22]. When further investigating the efficacy of tribendimidine against large cestodes, including *Taenia* spp. in humans, we propose to treat a small group of confirmed taeniasis cases who agreed to submit multiple stool samples and observe proglottids in their stools and underwear over extended time periods.

Infections with *A. lumbricoides* and hookworm are the main targets for single-dose mass chemotherapy using albendazole or mebendazole. Discussions are underway in the People's Republic of China for the larger scale use of tribendimidine. Efficacy of the latter drug on other intestinal parasites would be of considerable public health significance, which is explained by the geographic overlap of different helminth infections, including *S. stercoralis* and *Taenia* spp. Treatment of individuals with multiple species parasite infections, including *S. stercoralis* and *Taenia* spp., is likely to occur. Hence, there is a pressing need to determine the most efficacious dose of tribendimidine for treating *S. stercoralis* and *Taenia* spp. If parasites are exposed to sub-curative doses there is an elevated risk that tribendimidine resistance could develop. Therefore, pharmacovigilance needs to also cover non-target parasites to assure timely detection of emerging resistance.

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13. Discussion

This Ph.D. thesis pertains to the epidemiology, diagnosis and control of schistosomiasis japonica and intestinal helminthiasis in different settings of Yunnan province, China (chapters 8-12). In addition, the global estimates of the at-risk population and the number of infections due to schistosomiasis are updated, and the interplay with water resources development and management projects is examined (chapter 7). With regard to the Chinese work, the emphasis is on the regional scale, where we investigate the epidemiology and risk factors for *S. japonicum* and other helminth infections in Eryuan county, Yunnan province (Steinmann *et al.* 2007b), and put forward spatially-explicit prediction maps for *S. japonicum* seropositivity (Steinmann *et al.* 2007c). On the local scale, the epidemiology of intestinal multiparasitism (Steinmann *et al.* 2008a) and the endemicity of *S. stercoralis* in a selected community in the South of Yunnan province are elucidated (Steinmann *et al.* 2007a). Moreover, we explored the effect of sampling effort on the measured prevalence, and investigated the performance of various techniques for the diagnosis of intestinal parasites, with particular consideration to *S. stercoralis*. The last contribution is, to our knowledge, the first assessment of the safety and efficacy of tribendimidine against *S. stercoralis* and *Taenia* spp. (Steinmann *et al.* 2008b).

The justification for the design and implementation of these studies stems from both long-perceived research needs like the systematic assessment of the effects of water-related infrastructure on the local epidemiology of schistosomiasis and the study of the epidemiology of helminth infections in mountainous areas of western China, and conclusions drawn from recent investigations, e.g the possible activity of tribendimidine against further parasites including *S. stercoralis* and *Taenia* spp. The application of successful research approaches developed by members of our group in Côte d'Ivoire to a new setting in China was another request. The stratification of the individual projects according to the recently formulated strategic axes guiding the Swiss Tropical Institute (STI)-wide research, training and services activities – innovation, validation and application (STI 2006) – reveals a clear emphasis on innovation in a range of fields. Validation of previously established tools was achieved through the successful adaptation of a spatially-explicit Bayesian prevalence prediction model developed by Raso and colleagues (2005) for a hilly area in Côte d'Ivoire to a Chinese setting, and the performance assessment of different approaches for the diagnosis of intestinal parasites, notably *S. stercoralis*. The introduction of hitherto neglected diagnostic techniques to the helminthiasis department of the Simao branch of the Yunnan Institute for Parasitic Diseases rather belongs to the application domain of the STI strategy (Table 13.1).

Table 13.1 Stratification of the described studies according to the strategic axes formulated to guide the STI-wide activities.

Chapter	INNOVATION	VALIDATION	APPLICATION
7	The first quantification of (i) the population at risk of schistosomiasis due to water resources development and management, and (ii) of the associated risk ratios.		
8	The first integrated parasitological and serological helminth survey in a mountainous county in Yunnan province, China, including risk factor analysis.		
9		The adaptation of a spatially-explicit Bayesian statistical model integrating epidemiological and remotely-sensed environmental as well as geographical data to predict, for the first time, *S. japonicum* seroprevalences in a mountainous county in Yunnan province, China.	
10	The first comprehensive community-based investigation of intestinal multiparasitism in southern Yunnan province, China.		
10, 11			The introduction of sensitive diagnostic techniques (Baermann technique, Koga agar plate technique, SAF-conservation) for the evaluation of intestinal parasites in southern Yunnan, China and evaluation of the performance of these tools as well as the standard Kato-Katz technique under local conditions.
11	The establishment, for the first time, of the local endemicity of *S. stercoralis* in southern Yunnan province, China.		
12	The first assessment of the safety and efficacy of tribendimidine against *S. stercoralis* and *Taenia* spp. infections in humans.		

13.1 Water resources development and resulting effect on schistosomiasis

Theoretical considerations as well as obvious practical evidence suggest interactions between the construction and operation of dams and irrigation schemes and the local epidemiology of water-related vector-borne diseases like malaria, Japanese encephalitis and lymphatic filariasis, and water-based disease including schistosomiasis (Huang and Manderson 1992; Hunter *et al.* 1993; Jobin 1999). However, no attempt has been made before to systematically review the scientific literature for the respective evidence, and to put forward schistosomiasis-specific risk estimates related to the construction and operation of large dam reservoirs and surface irrigation schemes. To fill this gap we undertook in the framework of a WHO-supported project entitled "Burden of water-related vector-borne diseases: an analysis of the fraction attributable to components of water resources development and management" (Erlanger *et al.* 2005; Keiser *et al.* 2005a; 2005b).

We updated the global and country-specific estimates of the number of people infected or at risk of infection with schistosomes (Figure 13.1), and calculated that in mid-2003, 106 million out of globally 779 million (13.6%) individuals at risk of schistosomiasis lived adjacent (within 5 km) to large dam reservoirs or in areas under surface irrigation (Steinmann *et al.* 2006). The pooled random risk ratios for *S. haematobium* and *S. mansoni* were 2.4 (95% CI: 1.4-3.9) and 2.6 (95% CI: 1.4-5.0), respectively, for people living on the shores of reservoirs. With regard to proximity to irrigation schemes the risk for *S. haematobium* was 1.1 (95% CI: 0.02-7.3) and for *S. mansoni* it was 4.7 (95% CI: 0.49-23.0. No data pertaining to *S. japonicum* could be identified and this already triggered attention by other groups to investigate this issue (Li *et al.* 2007c). We further identified regional differences pertaining to the risk ratios and the frequency of schistosomiasis outbreaks in previously non-endemic areas following water resources development projects where it was found that *S. mansoni* was much more likely than *S. haematobium* to expand its endemic area following the implementation of water resources development projects.

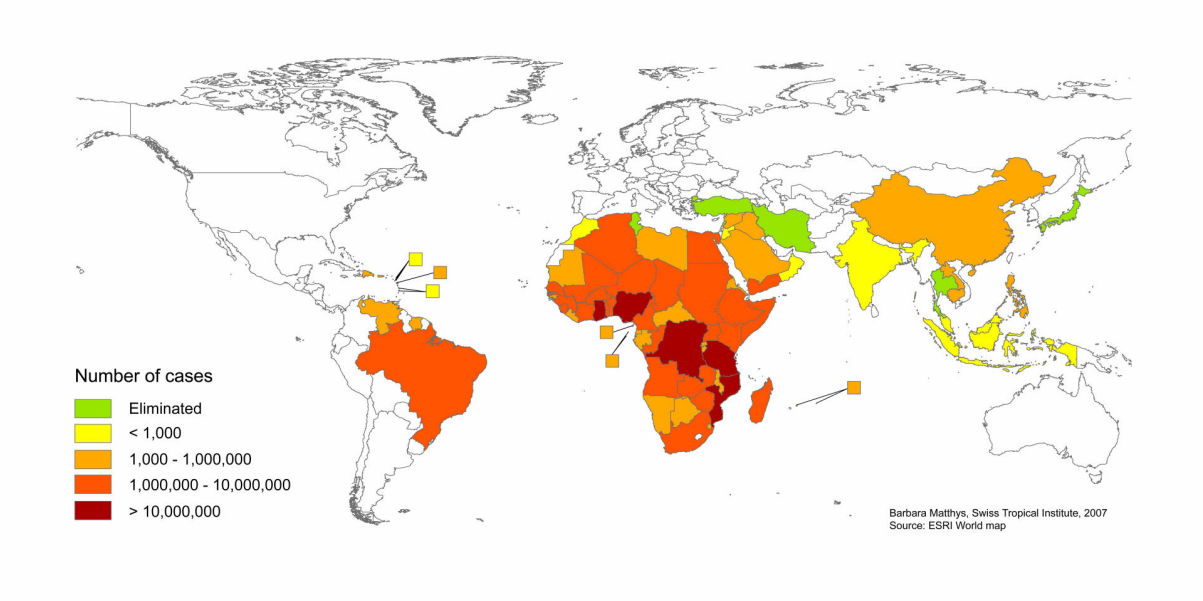

Figure 13.1 Number of schistosomiasis cases in mid-2003 in each endemic country. Data source: Steinmann *et al.* 2006; Appendix 7.2.

The data collection process indicated a dearth of contemporary country-specific estimates pertaining to schistosomiasis in Africa where an estimated 97% of the infected and 85% of the population at risk reside (Steinmann *et al.* 2006) and absolute numbers of parasitic infections are still increasing (de Silva *et al.* 2003). National surveys, e.g. modelled on the successful "nationwide cluster sampling survey on the epidemiology of schistosomiasis in the People's Republic of China" (Zhou *et al.* 2007) and adapted to the specific conditions of the respective country could provide these data. Another option is the integration of sound parasitological and epidemiological ground survey data and remotely-sensed environmental data, complemented by digital geographical maps, to construct comprehensive GIS databases for the display and query of spatial data (Brooker *et al.* 2000a; 2000b; 2002; Brooker and Michael 2000; Bergquist 2001; Yang *et al.* 2005; Rinaldi *et al.* 2006; Brooker 2007). The same databases also allow spatially-explicit modelling and prediction using advanced Bayesian statistical methods (Basáñez *et al.* 2004; Raso *et al.* 2006; Beck-Wörner *et al.* 2007; Brooker and Utzinger 2007). Equally transparent became the almost universal absence of data pertaining to small dams and informal irrigation, be it in Africa or elsewhere. It is conceivable that they exert at least equally profound effects on the local epidemiology of schistosomiasis but neither their numbers and extent nor their health-significance can currently be evaluated. The analysis of high-resolution RS imagery with sophisticated land use classification tools might provide the missing information on small-scale water resources development projects. Combined with ground-based epidemiological surveys, such a database might ultimately

allow the quantification of associated schistosomiasis risk ratios. We identified irrigation in sub-Sahara Africa as an area of particular concern since schistosomiasis is common in most of these countries and large-scale irrigation is not yet well developed in this region but might soon become so. Many regional countries still struggle to meet the dietary demands of their population, there is rapid population growth, and irrigation usually results in considerably increased productivity of agricultural land. Therefore, increased irrigation might be viewed as an obvious resort to boost food production. Already today, considerably informal irrigation and associated exacerbation of the local schistosomiasis transmission must be assumed.

Even if not complete, the available data pertaining to the effects of water resources development and management projects on different diseases strongly support recent calls for compulsory health impact assessment (HIA) to be integrated into the planning and implementation of respective projects (Singer and de Castro 2007; Erlanger *et al.* 2008). Mitigation activities funded from income derived from the ventures in question should become recognized standards, and the retrospective evaluation and setting-up of programmes to overcome negative health effects associated with already established projects are suggested.

13.2 *S. japonicum* and other helminths in a mountainous county of Yunnan province, China

S. japonicum arguably is one of the best-researched human parasite occurring in China. For decades, the schistosomiasis-related research and control activities focused on the vast endemic areas of the marshlands surrounding the great lakes of eastern China and along the Yangtze river, and on plain areas. Great progress in the study and control of this parasite has been achieved (Utzinger *et al.* 2005; Zhou *et al.* 2005). Meanwhile, little attention was paid to the endemic mountainous areas of southwest China (Chen and Feng 1999; Chen 2002). Difficult geographical conditions, slower socio-economic development, and lower numbers of affected people have been cited to explain this disregard (Chen 2002). However, it has long been acknowledged that future research should include mountainous areas to pay tribute to the distinct local conditions and to provide the scientific basis for locally-adapted control programmes (Chen 2002). Even less known is the epidemiology of other parasitic infections in mountainous areas despite clear evidence that the focus of soil-transmitted helminths has shifted westwards (Ministry of Health 2005). Moreover, other helminth infections including taeniasis, cysticercosis, trichinellosis and echinococcosis, are most prevalent in non-Han minority areas including mountainous parts of Sichuan and Yunnan provinces (Liu and

Boireau 2002; Chen *et al.* 2004; Li *et al.* 2005; Ministry of Health 2005; Cui *et al.* 2006). The probably most significant innovation of the cross-sectional survey in Eryuan county, however, is the concurrent parasitological investigation of *S. japonicum* and soil-transmitted helminths and serological screening of the population for schistosomiasis japonica, cysticercosis and trichinellosis using the local infrastructure established many years ago for surveillance and control of *S. japonicum*. Another novelty is the adaptation of a spatially-explicit statistical model for prevalence prediction developed for the hilly area of Man in western Côte d'Ivoire (Raso *et al.* 2005) to a mountainous area in China, and its application for *S. japonicum* seroprevalence risk profiling. The use of GIS, RS and spatial statistics in research and control of schistosomiasis japonica is well established (Zhou *et al.* 2001; Yang *et al.* 2005) but most applications focus on *O. hupensis* habitats or parasitological prevalences in plain and marshland areas. They often cover large study areas at the expense of spatial resolution (Yang *et al.* 2005).

The survey in the Bai- and Han-populated Eryuan county involved 3220 individuals aged 5-88 years and resulted in rather low egg-positive rates but high seroprevalences. The most common helminth was *A. lumbricoides* (15.4%), followed by *Taenia* spp. (3.5%) and *S. japonicum* in known endemic villages (2.7%). The highest seroprevalences were found for *Trichinella* spp. (58.8%) and *S. japonicum* in known endemic villages (49.5%). Cysticerci-specific antibodies were found in 18.5% of the study cohort. Important spatial heterogeneities of village-level prevalences as well as socio-economic conditions were observed. Identified risk factors for different infections included demographic (age, sex and ethnicity), socio-economic (asset-based wealth index and tobacco growing) and geographical (elevation and slope) indicators but trends were inconsistent, depending on the parasite. Thus, no homogeneous risk group for helminth infections could be identified but parasite-specific risk profiles were established (Steinmann *et al.* 2007b). The in-depth analysis of the seroprevalence data identified demographic (age and sex) and geographical (elevation and slope) risk factors for seroconversion and the prevalence prediction suggested high prevalence levels in low-lying plain areas which are mostly located in eastern Eryuan county (Steinmann *et al.* 2007c).

The evaluation of *S. japonicum* infections by both parasitological and serological methods in known *S. japonicum*-endemic, as well as in villages that were considered non-endemic identified several issues of considerable public health significance.

Firstly, seropositives and even egg-positive individuals were also identified in villages which were previously considered non-endemic for *S. japonicum*. This casts into doubt the

applicability of the standard strategy for the identification of endemic villages developed and successfully applied in the vast and more homogenous endemic areas of eastern China. According to the current approach, villages are first stratified into endemic and non-endemic ones based on identified *O. hupensis* habitats and known *S. japonicum* endemicity in humans, and further control efforts are directed at endemic villages only (Utzinger *et al.* 2005). The small-scale geographical heterogeneity and the often small and isolated snail foci in mountainous areas (Seto 2005) probably require different approaches for the identification of target areas for control. Further refined seroprevalence risk prediction models could offer a valuable tool to identify additional villages in need of schistosomiasis control.

Secondly, the current strategy to identify the target population for chemotherapy, i.e. serological screening followed by parasitological investigation of a single stool sample from the seropositives, probably becomes more unreliable as infection intensities decrease after many years of large-scale application of chemotherapy. We identified egg-positive individuals with negative serological results (sensitivity of serological pre-screening: 92.1%) and the collection and analysis of up to 3 stool samples instead of a single one lead to a markedly higher prevalence of egg-positive individuals. Thus, there is a pressing need for the development of more sensitive screening tests and, particularly, parasitological tests, and new ways for identification and spatial targeting of chemotherapy have to be explored. The miracidium hatching test is one possible alternative to the standard Kato-Katz technique but a recent study could not confirm its superiority over the former method (Yu *et al.* 2007). Another parasitological test with high potential is the FLOTAC method (Cringoli 2006) which is currently undergoing validation for *S. japonicum* diagnosis. Activities for the standardization of serological tests for schistosomiasis japonica have also been launched.

Thirdly, we found a pronounced difference between the seroprevalence and the egg-positive rate when using standard diagnostic tools. We speculate that this finding might indicate frequent exposure to infection and a preponderance of chemotherapy in the set of employed control tools. Chemotherapy effectively controls morbidity but neither prevents re-infection nor does it lead to sustainable control of *S. japonicum* (Utzinger *et al.* 2003; Liang *et al.* 2006; Singer and de Castro 2007). Therefore, the current control activities should be complemented by mechanization of agriculture, efforts for the control of *S. japonicum* in livestock, fenced pastures, and safe sanitation provided to special high-risk groups (e.g. fishermen) to break the transmission cycle (Wu *et al.* 2007). Sound health education and environmental management for snail control should also be emphasized. Thus, the national schistosomiasis control programme could become even more integrated and sustainable. On

the policy level, the national strategy needs to allow more flexibility and control approaches have to be adapted to local conditions since there are fundamental differences between endemic settings. Currently, a new national policy for integrated schistosomiasis control in China is under development and there is good prospects that the mentioned strategy adaptations will be considered (Wang *et al.* 2007a; 2007b).

The study has further shown that a host of helminths can be surveyed using the established infrastructure for *S. japonicum* surveillance and control. Even though the recorded average soil-transmitted helminth prevalences were low, the existing institutions can play a seminal role in delivering chemotherapy to heavily affected communities and to set up sustainable control programmes. *A. lumbricoides* was the most prevalent soil-transmitted helminth with highest prevalences recorded in poor population segments and remote areas, suggesting a direct link between socio-economic development and the prevalence of this parasite. Targeted control efforts could reduce the disease burden among the local poor, thus fostering the economic development in deprived areas (Molyneux and Nantulya 2004; Molyneux *et al.* 2005).

The seroprevalence of cysticercosis and trichinellosis was high. Both diseases are known to be endemic and emerging in Yunnan (Liu and Boireau 2002; Chen *et al.* 2004). There is an important focus in Dali prefecture but the local epidemiology of cysticercosis and trichinellosis remains to be further investigated. If the high prevalences are confirmed in additional studies, these findings are of considerable public health concern. While specialised treatment facilities have already been established in the regional center of Dali Xiaguan, the efforts for mapping, surveillance, and case detection in the field need to be increased. Health education, improved livestock husbandry, and comprehensive meat inspection are key to the control of trichinellosis and taeniasis, thus also contributing to cysticercosis control, while direct cysticercosis control mainly results from safe water supply, improved sanitation, and general hygiene (García *et al.* 2003). The ongoing socio-economic development of the study area is likely to both positively and negatively influence the prevalence of trichinellosis and cysticercosis. Water supply and sanitation usually improve in the face of economic development which also favours the establishment of formal food trade chains, improved livestock husbandry practices and institutions for meat inspection (Chen *et al.* 2004; Wang *et al.* 2006). Local traditions, on the other hand, cherish raw meat dishes and the increased purchase power of the population might result in broader availability and consumption of meat, especially in its prized raw form. A similar observation has been made for angiostrongyliasis in eastern China where a recent outbreak affected mainly well-off

inhabitants of Beijing (Lv *et al.* 2007). Our data reflect this ambivalent influence of the socio-economic development. The trichinellosis seroprevalence was higher among better-off population strata residing in plain areas who also profited from better access to clean water and sanitation. The (sero-)prevalence of *Taenia* spp. infection and cysticercosis, on the other hand, was higher among the poor who predominantly resided in mountainous areas where the provision of safe water and sanitation is still not ensured. However, the ongoing chemotherapy programme for schistosomiasis japonica control which mainly operates in the plain areas could also have contributed to this distribution pattern since *Taenia* spp. and cysticerci are highly sensitive to praziquantel treatment.

The high seroprevalences indicate almost area-wide endemicity of the investigated parasites, and ongoing exposure of sizeable population segments to respective sources of infection. This is of concern even in the absence of widespread morbidity and mortality owing to readily available chemotherapy. It must be suspected that any discontinuation of drug provision, for example as a result of resistance development or a massive economic downturn, would lead to a rapid increase in parasitological prevalences and overt morbidity.

13.3 Intestinal multiparasitism, *S. stercoralis* and their diagnosis in southern Yunnan

China is known for decades already to harbour about half of the global number of soil-transmitted helminth infections (Stoll 1947; de Silva *et al.* 2003) but still today, surprisingly little is known about the local epidemiology of different helminths. This dearth of reliable information is especially striking if only data in the (English) international literature are considered (Spear *et al.* 2004; Fung 2007; Liu *et al.* 2007). Ignoring the data from the first national sampling survey of human parasitic infections in China (Yu *et al.* 1994) which employed a diagnostic approach with a low sensitivity, i.e. direct smear, no epidemiological data pertaining to *S. stercoralis* in China have been published in neither Chinese nor English. Almost equally weak is the knowledge-base about intestinal protozoa and multiparasitism in general. The lack of a readily available diagnostic technique with a high sensitivity to detect *S. stercoralis* and intestinal protozoa in single stool samples as well as the local unavailability of further potent diagnostic techniques might be an important underlying reason. The paucity of information regarding the distribution and prevalence of intestinal parasites in China is disturbing and we speculate that the economic and public health significance of intestinal parasites across China might be considerably underestimated.

We introduced the Baermann (García 2007), Koga agar plate (Koga *et al.* 1991) and SAF-conservation technique (Marti and Escher 1990) to complement the current standard tool for stool examination in southern Yunnan province, i.e. the Kato-Katz technique (Katz *et al.* 1972). Using a rigorous diagnostic approach involving the collection of multiple stool samples per individual and their evaluation by different diagnostic techniques, we established the local endemicity of *S. stercoralis*, assessed the prevalence of further intestinal helminths and protozoa, and determined the multiparasitism rate in the village of Nongyang in southern Yunnan. The measured prevalence of *S. stercoralis* was 11.7% (Steinmann *et al.* 2007a) and the infection rate of the three most common soil-transmitted helminths exceeded 85% each. The prevalence of intestinal protozoa was generally low. Combined, 15 parasite species were identified, 8 helminths and 7 protozoa. Most study participants harboured 3 intestinal parasites concurrently (range: 1-6; Figure 13.2), and not a single individual without parasite infection was recorded (Steinmann *et al.* 2008a).

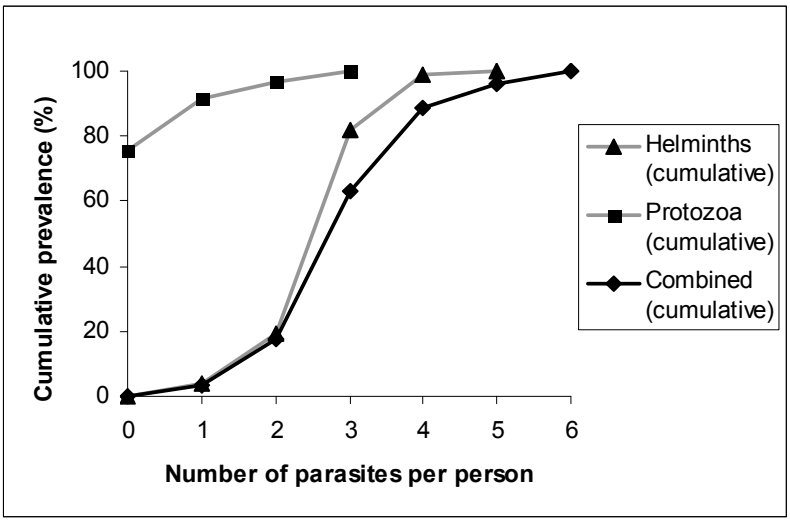

Figure 13.2 Cumulative prevalence of intestinal multiparasitism among 215 study participants from Nongyang village in Yunnan province, China.

The high prevalences indicate that MDA programmes urgently need to be initiated in the area to reduce morbidity and environmental contamination with helminth eggs. Control programmes that emphasis chemotherapy should later be complemented by more sustainable transmission control activities, including a strong health education component, provision of safe water supply and sanitation, and environmental management. The available data from Nongyang village indicate that latrine use and general hygiene behaviour remained weak even after the establishment of respective community facilities.

The data pertaining to eggs and larvae recovered during repeated stool sample collection were analysed by a mathematical model (Marti and Koella 1993). The results suggest a high sensitivity associated with the screening of three stool samples by either of the employed methods, corroborating previous statements about the recommended sampling frequency (van Gool *et al.* 2003). The effect was most notable for hookworm and *S. stercoralis*. The analysis of the collected samples by several diagnostic techniques also increased the measured prevalence of common soil-transmitted helminths, indicating imperfect sensitivity of the investigated diagnostic techniques even if the sample was positive. The sensitivity of intestinal protozoa detection in SAF-conserved samples by the ether-concentration method could not be evaluated since a single sample was analyzed by only one method. However, the screening of the same samples for *Blastocystis hominis* by a sensitive culture method and our approach, i.e. ether-concentration of SAF-conserved samples, indicated a prevalence of 32.6% with the former method (Li *et al.* 2007b) while it was 20.0% with the latter technique (Steinmann *et al.* 2008a), thus indicating considerable underdiagnosis using the ether-concentration technique.

There is a need for further evaluation of the performance of diagnostic tools and their standardization. Currently, it is often difficult to compare the results reported from different studies and settings due to divergent sample collection and processing standards and the use of different diagnostic approaches and criteria. Only rigorous sampling and the use of sensitive and reliable diagnostic tools allow the full appreciation of the local burden due to parasitic infections, and the unbiased evaluation of individual treatment or large-scale control activities irrespective of shifts in infection intensities (see chapter 4).

Our parasitological findings might be particular to the study area since Nongyang village is inhabited by a distinct ethnic group, i.e. Bulang. The socio-economic level and health-relevant infrastructure (water supply, sanitation, dispensaries, etc.) of the village are comparable to other Bulang villages but not to villages situated in plain areas and inhabited by other minorities, particularly Dai. However, there is a dearth of relevant data pertaining to the regional epidemiology of helminth infections and their association with distinct ethnic groups as well as environmental and socio-economic conditions. Further cross-sectional studies involving rigorous sampling and also serological testing for *S. stercoralis* are urgently needed in order to establish the true regional burden due to intestinal parasites.

The need for sound epidemiological data especially concerns *S. stercoralis* where no relevant information is currently available from China. Surveys are needed across the country to establish its true prevalence and the national public health significance of this neglected

parasite. The increased availability of sophisticated medical services, including immunosuppressive drugs and organ transplantation results in a growing number of immuno-compromised individuals in China – people who are at elevated risk of disseminated *S. stercoralis* infection if not diagnosed and treated in time. Systematic screening of those at risk using sensitive diagnostic techniques and prompt treatment of positives with efficacious drugs is crucial to avoid this severe and often fatal complication in already otherwise debilitated patients.

The studies also pointed out the limits of traditional parasitological investigations. The collection of multiple stool samples resulted in considerable discrepancies between the number of recruited study participants and the final cohort who had actually submitted multiple samples complying with the study requirements, i.e. were large enough to undergo multiple diagnostic tests. The screening of repeated samples per participant with several methods also translates into increased material, staff and monetary requirements. This calls for the development and validation of more sensitive but still economic diagnostic tools for the evaluation of intestinal parasites. The FLOTAC method (Cringoli 2006) holds promise to significantly improve the sensitivity of egg- and larvae detection in stool samples (Utzinger *et al.* 2008) and could become a new standard parasitological test. However, validation of this method for further parasites is still pending and experience with training of technicians and its use under field conditions are not yet available. The development of new tools for the detection of parasite DNA in stool using the PCR technology, and of parasite copro-antigens by specific antibodies should also be pushed ahead. Already established are respective tests for the diagnosis and differentiation of intestinal protozoa like *Entamoeba* spp. (Fotedar *et al.* 2007) and *Blastocystis hominis* (Li *et al.* 2007a) as well as various helminths, e.g. *Taenia* spp. and *Echinococcus* spp. (Mathis and Deplazes 2006; Craig and Ito 2007). In an interdepartmental collaboration, two groups at STI are currently developing a novel PCR-based diagnostic tool for *S. stercoralis* detection in fresh or frozen stool samples. Ultimately, such techniques might also allow the concurrent detection of different parasites.

13.4 Safety and efficacy of tribendimidine against *S. stercoralis* and *Taenia* spp.

The number of approved anthelminthic drugs is small and innovation in this field is very slow. This can be attributed to the good safety and therapeutic profiles against target parasites of available drugs, which set high standards for new compounds. Another reason are the high costs for research, development, testing and registration of new molecules which contrast with

the limited economic potential of such drugs, resulting in the abstinence of the established pharmaceutical industry from this field for many years. However, recent initiatives and innovative working arrangements involving non-governmental, supra-national and commercial partners (public-private partnerships, PPPs) have started to change this deplorable situation (Moran 2005).

Tribendimidine was developed by a research group in the National Institute of Parasitic Diseases in Shanghai from the early 1980s onwards, and its safety and efficacy for the treatment of common soil-transmitted helminth infections as well as *E. vermicularis* is well established. In early 2004, the drug was granted approval for human application by the State Food and Drug Administration of China (Xiao *et al.* 2005). Recent laboratory investigations also indicated activity of this compound against cestodes and *S. ratti* (Xiao *et al.* 2005; Keiser *et al.* 2008) but no trials in humans had yet been done. The experience related to tribendimidine treatment of individuals with multiple parasitic infections including other species than common soil-transmitted helminths is also limited.

We tested the safety and efficacy of a single 200 mg (5-14 year-old) or 400 mg (≥15 year-old) oral dose of tribendimidine in an open-label trial in Nanweng village in southern Yunnan where multiparasitism is pervasive in the local Bulang population. We focussed on the general safety and therapeutic effect on *S. stercoralis* and *Taenia* spp. investigated by a rigorous diagnostic approach involving the collection of multiple stool samples before and 2-4 weeks after treatment, and their analysis by sensitive diagnostic techniques. The results were compared to albendazole administered at standard doses. We found a *S. stercoralis*-specific cure rate of 54.5% ($P = 0.107$), *Taenia* spp. infections were cured in 66.7% of the study participants infected with this parasite ($P = 0.014$). Comparable prevalence reductions were achieved by single albendazole administration. At treatment evaluation, however, 'new' infections were found for both parasites and in both treatment groups, resulting in reduced net cure rates. This was particularly striking for *Taenia* spp. among those treated with albendazole and the difference between the drug-specific *Taenia* spp. net cure rates became highly significant ($P = 0.001$) (Steinmann *et al.* 2008b). Overall, tribendimidine was found to be safe for use in a setting where most individuals are infected with multiple intestinal helminth species concurrently, and the administration of a single standard dose of tribendimidine exhibited some effect on both *S. stercoralis* and *Taenia* spp., albeit at unsatisfactory levels. In a next step, the use of multiple, e.g. triple doses, is suggested and the effect should be compared to that of a standard drug against *S. stercoralis* and *Taenia* spp., i.e. ivermectin and praziquantel or niclosamide, respectively. Attention should also be paid to the

diagnostic technique, it is suggested to replace the microscopic *Taenia* spp. egg detection by copro-antigen ELISA and/or PCR, thus also allowing the differentiation of the *Taenia* species.

There is increasing evidence that single standard-dose treatment with common anthelminthic drugs (e.g. albendazole, mebendazole, praziquantel) can achieve high egg-reduction rates but often results in unsatisfactory clearance-of-infection levels. This observation particularly concerns *T. trichiura*; for tribendimidine it has been established that triple doses are needed to achieve high cure rates for this parasite (Xiao *et al.* 2005). The performance of single-dose albendazole to clear *T. trichiura* infections is better but mebendazole probably is not (Utzinger and Keiser 2004).

Worm burden and egg excretion reductions are important for morbidity control and result in decreased environmental egg contamination, thus easing the infection pressure. This provides the rationale guiding the ongoing large-scale MDA programmes which exclusively rely on single dose drug distribution (Fenwick 2006; Hotez *et al.* 2007). Financial and logistic constraints also favour this approach. However, there is growing awareness that these initiatives are unsustainable and long-term prevalence reductions or elimination can only be achieved by integrated control programmes considering water supply, sanitation, health education, environmental management, basic medical infrastructure, etc. (Utzinger *et al.* 2003; Singer and de Castro 2007), or similar changes brought along by sustainable and equitable socio-economic development.

MDA programmes rely on a mere handful of drugs, i.e. albendazole, mebendazole, levamisole, and pyrantel pamoate against major soil-transmitted helminths, praziquantel against schistosomes, and ivermectin or diethylcarbamazine for the treatment of onchocerciasis and lymphatic filariasis (Hotez *et al.* 2007). Used at standard doses, these drugs show excellent to satisfactory efficacy against the target parasites. Albendazole, for example, is highly active against *A. lumbricoides* if administered as a standard 400 mg single oral dose but the same dosage results in only moderate activity against *T. trichiura* (see above). In addition, most drugs also exhibit limited activity against a range of other parasites even if used at standard doses, e.g. against *S. stercoralis* and *Taenia* spp. as well as further helminths in the case of albendazole (Horton 2000; 2002). Acknowledging widespread intestinal polyparasitism (Buck *et al.* 1978; Keusch and Migasena 1982) and considering routine drug distribution to millions of individuals annually without prior diagnosis (Fenwick 2006; Molyneux 2006; Hotez *et al.* 2007), we would like to draw attention to some topics of considerable public health relevance.

Firstly, it must be suspected that co-treatment of non-target parasites is common, exposing at least some of them to sub-curative drug doses. An example is the use of albendazole at single doses in areas where *Taenia* spp. is endemic. This has implications for the development of drug resistance among human parasites, and necessitates resistance monitoring to include sympatric non-target parasites as well.

Secondly, co-treatment of secondary susceptible species could result in additional public health benefits, thus further raising the cost-effectiveness of these already highly successful interventions (Brady *et al.* 2006). Examples include *S. stercoralis* in areas covered by ivermectin-based MDA programmes and *Taenia* spp. in regions where praziquantel is distributed. Such added benefits of established programmes should be evaluated in order to provide accurate estimates of their real public health relevance, and to raise their appreciation among the concerned public and the donor community alike.

Thirdly, the selective efficacy of most drugs might arguably lead to shifts in the spectrum of endemic parasites in areas where MDA programmes are implemented over many years. It must be suspected that the relative public health importance of less susceptible parasites increases, e.g. *S. stercoralis* and also *T. trichiura* in areas covered by albendazole or mebendazole MDA programmes against soil-transmitted helminths but not in areas where ivermectin-based onchocerciasis control is implemented.

13.5 Conclusions

We conclude that water resources development projects can seriously impact on the local epidemiology of schistosomiasis, and that a large number of people is currently exposed to an elevated schistosomiasis infection risk due to the construction and operation of such facilities. We further conclude that the epidemiology of *S. japonicum* and soil-transmitted as well as food-borne helminths in the mountainous Eryuan county of Yunnan province in China is governed by complex demographic, socio-economic, and environmental risk factors and is likely to change due to rapid shifts in economic conditions. Integrating various data sources, the prediction of the *S. japonicum* seroprevalence is feasible but respective models need further improvement to assist the control programme. Control strategies have to respond to these challenges and should focus on general parasitic infections rather than *S. japonicum* alone. In southern Yunnan, soil-transmitted helminth infections and intestinal multiparasitism are rampant, at least in distinct communities, and *S. stercoralis* is endemic. This requires immediate implementation of morbidity control programmes, further surveys using sensitive approaches, and the establishment of a system for monitoring and surveillance. The performance of standard diagnostic tools for the detection of intestinal helminth infections is good if multiple stool samples are collected. Further investigations pertaining to the efficacy of tribendimidine for the treatment of intestinal parasitic diseases with a focus on *S. stercoralis* and *Taenia* spp. are warranted.

The formulation of sensible national parasite control strategies is challenging in China due to the climatic, geographical and socio-economic heterogeneity which is mirrored in largely divergent epidemiological situations across the country. Policies need to be flexible enough to allow their adaptation to locally prevailing conditions, and tools for control have to respond to local needs and realities. Last, innovative funding strategies have to be developed to cope with the unequal distribution of the burden posed by parasitic infections and the economic potential of local, regional and provincial authorities.

13.6 Identified research needs

- Quantification of small reservoirs and informal irrigation with a focus on sub-Saharan Africa; their effect on the local epidemiology of schistosomiasis and other diseases.
- Effects of water resources development on the local epidemiology of *S. japonicum* and other diseases in China.
- Development of a reliable screening strategy to identify *S. japonicum*-endemic communities in mountainous areas; further establishment of the epidemiology of *S. japonicum*, soil-transmitted helminths and food-borne helminths in mountainous areas.
- Further adaptation of *S. japonicum* seroprevalence prediction models to mountainous settings and ground-validation of the prediction.
- Evaluation of the effects of socio-economic conditions (and changes therein) on the local epidemiology of schistosomiasis japonica, soil-transmitted as well as food-borne helminths, and intestinal multiparasitism in China.
- Spatial distribution and local epidemiology (risk factors, transmission sites, reservoirs) of *S. stercoralis*, intestinal protozoa, and intestinal multiparasitism in China.
- Further development and/or validation of sensitive diagnostic tools for the evaluation of intestinal multiparasitism, e.g. the FLOTAC technique, with an aim to establish acknowledged diagnostic standards.
- Design and cost-effectiveness assessment of safe and efficacious treatment strategies in settings where intestinal multiparasitism is pervasive.
- Evaluation of the safety and efficacy of revised tribendimidine treatment schemes for *S. stercoralis* and *Taenia* spp.; evaluation of the safety and efficacy of tribendimidine against additional helminths and intestinal protozoa.
- Assessment of the performance of microscopic *Taenia* spp. diagnosis from multiple stool samples, e.g. using coproantigen ELISA or PCR; differentiation of *Taenia* spp. in Yunnan province, China to the species level using PCR.
- Assessment of ancillary benefits of MDA, namely on non-target parasites.

13.7 Recommendations

- Introduction of compulsory health impact assessment accompanying all water resources development projects in tropical countries; enforcement of adequate mitigation programmes.
- Broaden the mandate of the established infrastructure for *S. japonicum* control to include (intestinal) helminths in general.
- Adaptation of the national schistosomiasis control programme in China to (i) provide for increased local adaptation of general recommendations, (ii) account for the reduced sensitivity of traditional serological and parasitological methods for *S. japonicum* diagnosis following repeated rounds of chemotherapy, and (iii) recognize the small-scale heterogeneity of endemicity levels in mountainous areas and presence of infections at some distance from transmission sites.
- Systematic assessment of intestinal multiparasitism (at least helminths) in the course of all screening activities involving the collection of stool samples across China.
- Establishment of meat inspection services for the control of *Taenia* spp. and *Trichinella* spp.
- Launch of MDA programmes for the control of soil-transmitted helminths and *Taenia* spp. in high-endemicity areas of Yunnan province. Establishment of integrated control programmes.
- Introduction of systematic *S. stercoralis* screening of all individuals undergoing immunosuppressive drug therapy or diagnosed with relevant infections in China; prompt treatment of those found to be infected.

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14. Curriculum Vitae

Personal data

Full name	Peter Jakob Steinmann
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Marital status	Single
Nationality	Swiss
Languages	German (Mother tongue), English (very good), French (good), Italian (basic)
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Education and work experience

12.2004 – 11.2007	**Ph.D. in Epidemiology** Swiss Tropical Institute (STI) Department of Public Health and Epidemiology
Ph.D. thesis	Epidemiology and diagnosis of *Schistosoma japonicum*, other helminth infections and multiparasitism in Yunnan province, China Supervision: Prof. Dr. Jürg Utzinger (STI), Prof. Dr. Marcel Tanner (STI), Dr. Hanspeter Marti (STI), Dr. Robert Bergquist (retired from WHO/TDR), Prof. Dr. Zhou Xiaonong (National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China)
11.2003 – 10.2004	**Trainee** RCC Ltd. Itingen/Füllinsdorf, Switzerland Departments of Toxicology/Safety Assessment + Regulatory Affairs
10.1998 – 7.2003	**M.Sc. (Diploma) in Biology I** (Integrative Biology) University of Basel & Swiss Tropical Institute (STI)
M.Sc. thesis	Brucellosis and Q-fever in Mali: Case detection, role of milk contamination and other risk factors Supervision by Prof. Dr. Jakob Zinsstag (STI) and Dr. Bassirou Bonfoh (STI)
8.1995 -12.1997	**High School** Gymnasium Oberwil, Switzerland
Emphasis	Latin

Oral presentations at scientific meetings

10.09.2007	Joint meeting 7th RNAS+ Workshop/1st International Symposium on Geospatial Health Regional Network of Asian Schistosomiasis and other Helminth Zoonoses Global Network for Geospatial Health Lijiang, People's Republic of China (05.-10.09.2007) 1. Occurrence, risk factors and spatial distribution of helminth infections in Eryuan county, Yunnan province, China 2. Diagnosis of intestinal helminths and protozoa with particular consideration to Strongyloides stercoralis: Evaluation of multiparasitism in rural China
23.09.2006	Joint meeting SSTMP/RSTM&H Swiss Society of Tropical Medicine and Parasitology/Royal Society of Tropical Medicine and Hygiene, Basel, Switzerland (22.-23.09.2006) Dams, irrigation and disease: Effects of water resources development on schistosomiasis
05.09.2006	BA Festival of Science British Association for the Advancement of Science, Norwich, United Kingdom (02.-09.09.2006) Dams, irrigation and disease: Effects of water resources development on schistosomiasis
10.08.2005	5th RNAS Workshop Regional Network on Asian Schistosomiasis, Bali, Indonesia (08.-10.08.2005) Systematic literature review and meta-analysis on the effect of water resources development and management on schistosomiasis

Funding

2006	Personal stipend	Janggen-Pöhn Stiftung, St. Gallen, Switzerland (CHF 25,000.-)
	Project grant	Commission for Research Partnership with Developing Countries (KFPE), through the SDC-sponsored programme „Jeunes Chercheurs"), Bern, Switzerland (CHF 35,000.-)
	Project grant	Freiwillige Akademische Gesellschaft, Basel, Switzerland (CHF 15,000.-)

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