

**Epidemiology and diagnosis of schistosomiasis in
preschool-aged children in Azaguié, south Côte d'Ivoire**

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Summary

Background: Classified among the neglected tropical diseases (NTDs), schistosomiasis remains one of the most important parasitic diseases in the tropics and subtropics, and constitutes a major public health problem. Following World Health Assembly (WHA) resolution 54.19, put forth in May 2001, several control programmes have emerged in schistosomiasis-endemic countries with the objective to reduce morbidity due to schistosomiasis and soil-transmitted helminthiasis by regularly treating at least 75% and up to 100% of all school-aged children who are at risk by 2010. By focusing treatment upon the school-aged population, WHA resolution 54.19 neglects preschool-aged children, thus preventing them from benefiting from preventive chemotherapy targeted to their older peers, and hence creating a potential health inequity. Root causes include the belief that very young children would not yet be exposed to infected freshwater bodies, thus an insufficient understanding and documentation of the extent and severity of schistosomiasis in this age class, and a paucity of pharmacokinetic safety data of praziquantel among young children. However, in endemic zones, women are frequently accompanied by their children, even at young age, when they go to ponds, rivers or irrigation canals, all of which may be contaminated with cercariae, the infective stage to humans. Recent studies carried out in East and West Africa showed that intestinal and urogenital schistosomiasis can indeed occur in very early childhood. Pathology due to chronic infection with *Schistosoma mansoni* includes hepatic perisinusoidal egg granulomas, Symmers' pipe-stem periportal fibrosis, portal hypertension and, occasionally, embolic egg granulomas in the brain or spinal cord. *Schistosoma haematobium* infection may cause haematuria, scarring, calcification, squamous cell carcinoma and, occasionally, embolic egg granulomas in the brain or spinal cord.

Goal and specific objectives: The overarching goal of this Ph.D. thesis is to deepen our understanding of the epidemiology of schistosomiasis in preschool-aged children. The thesis pursued five specific objectives in Azaguié district, south Côte d'Ivoire. First, to characterize intestinal parasitic infections at the Azaguié district level. Second, to assess the accuracy of a commercially available urine circulating cathodic antigen (CCA) cassette test (CCA-A) and an experimental formulation (CCA-B) for the diagnosis of *S. mansoni* among school-aged children in different endemicity settings. Third, to assess the accuracy of CCA-A for the diagnosis of *S. mansoni* in preschool-aged children before and after praziquantel administration. Fourth, to study the epidemiology and risk factors for schistosomiasis in

preschool-aged children. Fifth, to assess the efficacy and safety of crushed praziquantel tablets in preschool-aged children in a co-endemic setting of *S. mansoni* and *S. haematobium*.

Methods: The fieldwork for this Ph.D. thesis was split into two parts. In order to address the first two objectives, in mid-2010, a cross-sectional study was carried out in seven schools from four locations of Azaguié district, including more than 600 schoolchildren. Multiple stool and urine samples were collected from each schoolchild over three consecutive days. Stool samples were examined with the Kato-Katz technique for the diagnosis of *S. mansoni* and soil-transmitted helminths (*Trichuris trichiura*, *Ascaris lumbricoides* and hookworm). Stool samples from the first day of collection were preserved in sodium acetate-acetic acid-formalin (SAF) and examined one month later using an ether-concentration method for the diagnosis of intestinal protozoa. Urine samples were examined with CCA tests (CCA-A on three days and CCA-B once) for the diagnosis of *S. mansoni*. In addition, urine samples were analysed with the urine filtration technique and reagent strips for the diagnosis of *S. haematobium*.

In order to address objectives 3-5, a cross-sectional study was implemented as a baseline survey in 2011 in two villages of Azaguié district, namely Azaguié Makouguié and Azaguié M'Bromé, where *S. mansoni* and *S. haematobium* coexist. About 300 preschool-aged children (<6 years) were involved in this study. Multiple stool and urine samples were collected over two consecutive days and subjected to the same laboratory procedures as the samples of the schoolchildren in 2010. Anthropometric measures (weight, height and arm circumference) and clinical features (temperature, haemoglobin level) from each preschool-aged child were recorded. Focus group discussions were performed with the mothers of the preschool-aged children and questionnaires administered for a risk factor assessment. Subsequently, preschool-aged children were treated with crushed praziquantel tablets and three weeks posttreatment, drug efficacy was determined following the same field and laboratory procedures as during the baseline study. Adverse events (within 3 and 24 hours posttreatment) were recorded by interviewing the mothers of the preschoolers.

Results: The results of this PhD thesis can be structured as follows:

Intestinal parasitic infections in Azaguié: We showed that the selection of intervention settings by control programmes based on a single stool sample examined with duplicate Kato-Katz thick smears or a single urine sample subjected to a standard urine

filtration method considerably underestimate the prevalence of *Schistosoma* infection. This led to a misclassification of intervention settings as defined by the World Health Organization (WHO) guidelines. Hence, in such a context, more sensitive diagnostic tools are needed to select the intervention settings with high accuracy. In addition, we found a small-scale heterogeneity in the distribution of helminth and intestinal protozoa infections. We also confirmed that polyparasitism is common in the Azaguié district.

Accuracy of urine CCA tests in different endemicity settings in schoolchildren:

The prevalence of *S. mansoni* in the three different endemicity settings was 32.9%, 53.1% and 91.8%, respectively. In all three settings, the sensitivity of a single CCA-A test was similar to triplicate Kato-Katz thick smears and was 56.3% and 47.9% in setting A (*S. mansoni* prevalence, 32.9%), 69.6% and 73.9% in setting B (*S. mansoni* prevalence, 53.1%), and 89.6% and 94.2% in setting C (*S. mansoni* prevalence, 91.8%). The specificity of the CCA-A test was moderate (76.9–84.2%). The likelihood of a CCA-A test color reaction increased with higher *S. mansoni* faecal egg counts (odds ratio = 1.07, $p < 0.001$). A concurrent *S. haematobium* infection or the presence of microhaematuria did not influence the CCA test results for *S. mansoni* diagnosis.

Accuracy of the urine CCA test in preschool-aged children: Before treatment, the prevalence of *S. mansoni*, as determined by quadruplicate Kato-Katz thick smears, duplicate CCA(t-) test considering “trace” as negative results, and CCA(t+) test with “trace” as positive, was 23.1%, 45.0% and 76.5%, respectively. Irrespective of the ‘gold’ standard, a single CCA test (CCA(t+) or CCA(t-)) was more sensitive than quadruplicate Kato-Katz thick smears before and after treatment. The specificity of a single CCA test ranged between 59.3% and 100% before and after treatment. The intensity of the CCA test band reaction was correlated with *S. mansoni* egg burden (odds ratio = 1.2, $p = 0.04$).

Epidemiology and risk factors of schistosomiasis in preschoolers: The prevalence of *S. mansoni* in preschool-aged children was 25.5% in Azaguié Makouguié and 21.6% in Azaguié M’Bromé and the prevalence of *S. haematobium* 17.3% and 5.9%, respectively. Most infections were of light intensity. Mothers’ occupation and older siblings played important roles in the epidemiology of schistosomiasis in preschool-aged children.

Efficacy and safety of crushed praziquantel in preschoolers: According to the Kato-Katz and urine filtration results, we found high efficacy of crushed praziquantel against *S. mansoni* (cure rate (CR) = 88.6%, egg reduction rate (ERR) = 96.7%) and *S. haematobium* (CR = 88.9%, ERR = 98.0%). Treatment was generally well tolerated, but moderate adverse

events (i.e. body and face inflammation), which required close supervision by the study physician, were observed in four non-infected children.

Conclusions: More sensitive diagnostic tools and rigorous sampling approaches are needed to select schistosomiasis-endemicity settings with high accuracy. The observed small-scale heterogeneity of helminth and intestinal protozoa infections should be carefully considered by control programmes. A single urine CCA test is more sensitive than multiple Kato-Katz thick smears in school-aged as well as in preschool-aged children. The urine CCA test can be recommended for rapid identification of high risk communities. However, its application for monitoring the impact of control interventions needs further investigation. In our study settings, preschool-aged children are at risk of schistosomiasis and can be infected very early in childhood. Integrated control approaches including improvement of safe water supply, sanitation, health facilities, and health education are needed in our study communities. Crushed praziquantel is efficacious against *S. mansoni* and *S. haematobium* and can be recommended for the treatment of infected children at young age, but only if they are unambiguously diagnosed. Nevertheless, further research is needed to deepen our understanding on the safety of praziquantel in this age group.

Zusammenfassung

Hintergrund: Klassifiziert als vernachlässigte Tropenkrankheit, bleibt Schistosomiasis eine der wichtigsten parasitären Krankheit in den Tropen und Subtropen, und stellt ein bedeutendes Problem für die jeweilige Volksgesundheit dar. Im Anschluss an die World Health Assembly (WHA) Resolution 54.19 vom Mai 2001 entstanden mehrere Kontrollprogramme in endemischen Gebieten mit dem Ziel die durch Schistosomiasis verursachte Morbidität zu reduzieren, indem mindestens 75% aller Schulkinder bis 2010 behandelt werden. Durch die Fokussierung auf Schulkinder vernachlässigt die WHA Resolution 54.19 aber Kinder im Vorschulalter, wodurch diese nicht von den Praziquantel Behandlungen der älteren Kinder profitieren und möglicherweise eine Gesundheitsungleichheit geschaffen wird. Gründe für diese Fokussierung liegen in dem Glauben, dass ganz junge Kinder nicht infizierten Süßwassergewässern ausgesetzt sind, sprich in einem ungenügenden Verständnis und einer mangelnden Dokumentation vom Ausmass und der Schwere von Schistosomiasis in dieser Altersgruppe, und dem Mangel an Daten zur pharmakokinetischen Sicherheit von Praziquantel in jungen Kindern. Frauen nehmen jedoch häufig ihre noch sehr jungen Kinder mit, wenn sie zu den Teichen, Bächen oder Bewässerungskanälen gehen. Diese können mit Zerkarien kontaminiert sein, dem für den Menschen infektiösen Parasitenstadium. Neuere, vor allem in Ost- und Westafrika durchgeführte Studien haben gezeigt, dass intestinale und urinäre Schistosomiasis tatsächlich schon in frühester Kindheit auftreten kann. Der Krankheitsverlauf von chronischen Infektionen mit *Schistosoma mansoni* kann hepatische, perisinusoidale Granuloma wegen der Parasiteneier, Symmers' pipe-stem periportal fibrosis, portale Hypertonie und gelegentlich embolische Granuloma wegen der Parasiteneier im Gehirn oder Rückenmark einschliessen. Infektionen mit *Schistosoma haematobium* können Hämaturie, Narbenbildung, Verkalkung, Plattenepithelkarzinom und gelegentlich ebenfalls embolische Granuloma wegen der Parasiteneier im Gehirn oder Rückenmark verursachen.

Ziele: Die vorliegende Dissertation verfolgte fünf spezifische Ziele im Azaguié Distrikt im Süden der Côte d'Ivoire. Erstens, die Charakterisierung von intestinalen Parasiteninfektionen im Azaguié Distrikt. Zweitens, die Beurteilung der Genauigkeit von einem kommerziell erhältlichen sogenannten „urine circulating cathodic antigen (CCA) cassette test“ (CCA-A) und einem Test mit einer experimentellen Formulation (CCA-B) für die Diagnose von *S. mansoni* bei Schulkindern in verschiedenen endemischen Gebieten. Drittens, die

Beurteilung der Genauigkeit vom CCA-A Test für die Diagnose von *S. mansoni* in Vorschulkindern vor und nach einer Praziquantel-Behandlung. Viertens, das Beschreiben der epidemiologischen Situation und Risikofaktoren für Schistosomiasis in Vorschulkindern. Fünftens, die Beurteilung der Wirksamkeit und Sicherheit von zerdrückten Praziquantel-Tabletten bei der Behandlung von Vorschulkindern in einem Gebiet, das co-endemischen ist für *S. mansoni* und *S. haematobium*.

Methoden: Die Feldarbeit für die vorliegende Dissertation wurde in zwei Teile aufgeteilt. Um die ersten zwei Ziele anzugehen wurde 2010 eine Querschnittstudie in sieben Schulen in vier verschiedenen Orten im Azaguié Distrikt durchgeführt, welche über 600 Schulkinder einschloss. Während drei aufeinanderfolgenden Tagen wurden mehrere Stuhl- und Urinproben von den Schulkindern gesammelt. Die Stuhlproben wurden mit der Kato-Katz Technik nach *S. mansoni* und nach durch Bodenkontakt übertragenen parasitischen Würmern (*Trichuris trichiura*, *Ascaris lumbricoides* und Hakenwürmer) untersucht. Stuhlproben vom ersten Tag wurden in Sodium Acetate-Acetic Acid-Formalin (SAF) konserviert und einen Monat später mit der Ether-Konzentrations-Methode nach Darmprotozoen untersucht. Die Urinproben wurden mit den CCA Tests (CCA-A und CCA-B) auf *S. mansoni* getestet. Zusätzlich wurden die Urinproben mit der Urine-Filtrations-Methode und mit Reagenzstreifen analysiert um *S. haematobium* zu diagnostizieren.

Um die Ziele drei, vier und fünf anzugehen wurde 2011 eine Querschnittstudie als Baseline-Studie in den zwei Dörfern Azaguié Makouguié und Azaguié M'Bromé des Azaguié Distrikt durchgeführt. In den beiden Dörfern treten sowohl Infektionen mit *S. mansoni* als auch *S. haematobium* auf. Ungefähr 300 Vorschulkinder (<6 Jahre) wurden in der Studie berücksichtigt. Während zwei aufeinanderfolgenden Tagen wurden mehrere Stuhl- und Urinproben gesammelt und mit denselben Laboranalysen untersucht wie die Proben der Schulkinder im Jahr 2010. Anthropometrische Masse (Gewicht, Grösse und Armumfang) und klinische Aspekte (Temperatur und Hämoglobin-Level) wurden von jedem Vorschulkind aufgezeichnet. Fokusgruppengespräche wurden mit den Müttern der Vorschulkinder durchgeführt und ein Fragebogen ausgeteilt zur Erfassung von Risikofaktoren. Anschliessend wurden die Vorschulkinder mit zerdrückten Praziquantel-Tabletten behandelt und drei Wochen später der Effekt der Behandlung mit denselben Feld- und Labor-Methoden beurteilt, wie während der Baseline-Studie. Nebenwirkungen wurden mittels Interviews mit den Müttern der Vorschulkinder erfasst.

Ergebnisse: Die Ergebnisse dieser Dissertation können folgendermassen zusammengefasst werden:

Intestinale Parasiteninfektionen in Azaguié: Unsere Ergebnisse zeigen, dass die Auswahl von Interventionsgebieten von Kontrollprogramme aufgrund von nur einer Stuhl- oder Urinprobe, welche mit doppelten Kato-Katz Test bzw. einer einzelnen Urine-Filtration analysiert werden, problematisch ist, da die entsprechenden Schistosomiasis-Prävalenzen in der Regel unterschätzt werden. Dies führte zu einer falschen Klassifizierung der Interventionsgebiete gemäss Richtlinien der Weltgesundheits-organisation (WHO). Daher werden sensitivere Diagnostiktests benötigt um Interventionsgebieten mit hoher Präzision auszusuchen. Zudem wurde eine kleinräumige Heterogenität in der Verteilung von intestinalen Wurm- und Protozoeninfektionen festgestellt. Unsere Ergebnisse bestätigten auch, dass Polyparasitismus häufig vorkommt im Azaguié Distrikt.

Genauigkeit von CCA Tests bei Schulkindern in verschiedenen endemischen Gebieten: Die Prävalenz von *S. mansoni* in den drei unterschiedlich endemischen Gebieten betrug 32.9%, 53.1% und 91.8%. Die Sensitivität von einem einzelnen CCA-A Test war vergleichbar mit dreifachen Kato-Katz Tests in allen drei Gebieten. Die Spezifität des CCA-A Tests war moderat (76.9–84.2%). Die Wahrscheinlichkeit einer Farbreaktion beim CCA-A Test war höher mit einer höheren Anzahl von *S. mansoni* Eiern in den Stuhlproben (Odds Ratio = 1.07, $p < 0.001$). Eine gleichzeitige Infektion mit *S. haematobium* oder das Auftreten einer Mikrohämaturie beeinflusste die CCA Testresultate für *S. mansoni* nicht.

Genauigkeit von CCA Tests bei Vorschulkindern: Vor der Behandlung war die Prävalenz von *S. mansoni* 23.1%, 45.0% und 76.5%, gemäss vierfachen Kato-Katz Tests, doppelten CCA(t-) Tests („traces“ als negative Ergebnisse) und einem CCA(t+) Test („traces“ als positive Ergebnisse). Ein einzelner CCA Test (CCA(t-) oder CCA(t+)) war sensitiver als vierfache Kato-Katz Tests, sowohl vor als auch nach der Behandlung, und unabhängig vom jeweiligen Goldstandard. Die Spezifität eines einzelnen CCA Tests bewegte sich vor und nach der Behandlung zwischen 59.3% und 100%. Die Intensität der Testbandreaktion korrelierte mit der Anzahl *S. mansoni* Eier (Odds Ratio = 1.2, $p = 0.04$).

Epidemiologie und Risikofaktoren für Schistosomiasis bei Vorschulkindern: Die *S. mansoni* Prävalenz in Vorschulkindern betrug 21.6% in Azaguié M'Bromé und 25.5% in Azaguié Makouguié und die *S. haematobium* Prävalenz 5.9% und 17.3%. Die Intensität der meisten Infektionen war gering. Die Beschäftigung der Mütter und die älteren Geschwister spielten eine wichtige Rolle in der Epidemiologie von Schistosomiasis bei den Vorschulkindern.

Wirksamkeit und Sicherheit von zerdrückten Praziquantel-Tabletten bei Vorschulkindern: Basierend auf den Resultaten der Kato-Katz und Urin-Filtration Tests, stellten wir eine hohe Wirksamkeit von zerdrückten Praziquantel-Tabletten gegen *S. mansoni* (Heilungsrate (CR) = 88.6%, Eireduktionsrate (ERR) = 96.7%) und *S. haematobium* (CR = 88.9%, ERR = 98.0%) fest. Die Verträglichkeit der Behandlung war im Allgemeinen gut, aber moderate Nebenwirkungen (Entzündungen am Körper und im Gesicht) wurden bei vier nicht-infizierten Kindern beobachtet, welche eine genauere Überwachung durch den Studienarzt erforderten.

Schlussfolgerung: Sensitivere Diagnostikmethoden und eine rigorose Stichprobenauswahl sind nötig um bestimmte endemische Gebiete mit hoher Präzision auszusuchen. Die festgestellte kleinräumige Heterogenität von intestinalen Wurm- und Darmprotozoen infektionen sollte von Kontrollprogrammen sorgfältig berücksichtigt werden. Ein einzelner CCA Test ist sensitiver als mehrfache Kato-Katz Tests, sowohl bei Schulkindern als auch bei Vorschulkindern. Der CCA Test kann zur raschen Identifikation von Hochrisiko-Gemeinschaften empfohlen werden. Seine Anwendung zur Überwachung von Kontrollinterventionen braucht aber noch weitere Abklärungen. In unserem Studiengebiet besteht für Vorschulkinder das Risiko an Schistosomiasis zu erkranken und erste Infektionen treten bereits in frühester Kindheit auf. Integrierte Ansätze zur Kontrolle sind nötig in den untersuchten Gemeinschaften und beinhalten eine verbesserte Versorgung mit sauberem Wasser, bessere sanitäre Einrichtungen, bessere Gesundheitseinrichtungen und eine gesundheitliche Aufklärung. Zerdrückte Praziquantel-Tabletten sind wirksam gegen *S. mansoni* und *S. haematobium* und können zur Behandlung von infizierten Kindern empfohlen werden, aber nur, wenn sie vorher eindeutig diagnostiziert worden sind. Weitere Untersuchungen sind auf jeden Fall noch nötig um unser Verständnis zur Sicherheit von Praziquantel in dieser Altersgruppe zu vertiefen.

Résumé

Contexte: Classé parmi les maladies tropicales négligées (MTN), la schistosomiase demeure une des maladies parasitaires les plus importantes dans les régions tropicales et subtropicales, et constitue un problème majeur de santé publique. Suite à la résolution 54.19 de l'Assemblée Mondiale de la Santé (AMS) mise en avant en mai 2001, plusieurs programmes de contrôle ont fait leur apparition dans les zones endémiques avec pour objectif principale de réduire la morbidité due à la schistosomiase en traitant au moins 75% de tous les enfants d'âge scolaire des zones endémiques jusqu'en 2010. En se focalisant sur le traitement de la population d'âge scolaire, la résolution WHA 54.19 néglige de facto les enfants d'âge préscolaire, les empêchant ainsi de bénéficier du traitement par le praziquantel, d'où la naissance d'une inégalité potentiel quant à leur la santé. Les causes profondes seraient entre autres la croyance à la non exposition des plus jeunes enfants à des organismes d'eaux douces infectées, le manque de compréhension et de documentation sur l'étendue et la gravité de la schistosomiase dans cette classe d'âge et un manque de données sur la sécurité pharmacologique du praziquantel chez les jeunes enfants. Toutefois, dans les zones endémiques, les femmes sont souvent accompagnées de leurs enfants (même à un jeune âge), quand elles vont dans les étangs, les rivières ou les canaux d'irrigation. Ceux-ci peuvent abriter des cercaires, le stade infestant pour les humains. Des études récentes mises en œuvre principalement à l'Est et l'Ouest de l'Afrique ont montré que la schistosomiase intestinale ou urinaire peut en effet survenir dans la petite enfance. Les pathologies liées à une infection chronique par *Schistosoma mansoni* sont entre autres, les granulomes hépatiques périsinusoïdales d'oeufs, les fibroses périportales du canal de Symmers, les hypertensions portales, et parfois les granulomes d'oeufs conduisant à des embolies dans le cerveau ou la moelle épinière. Celles causées par *S. haematobium* comprennent les hématuries, les cicatrices, les calcifications, les carcinomes à cellules squameuses et, occasionnellement, des embolies dans le cerveau ou la moelle épinière due aux granulomes d'oeufs.

Objectifs: Cette thèse de doctorat a poursuivi cinq objectifs spécifiques dans le district d'Azaguié, sud de la Côte d'Ivoire. Nous visons premièrement à bien caractériser les parasitoses intestinales au niveau du district Azaguié. Deuxièmement, à évaluer dans des zones d'endémicité différentes (A, B, C), la fiabilité d'un test immunologique, disponible dans le commerce (désigné CCA-A) et d'une formulation expérimentale (désigné CCA-B) dans le diagnostic *S. mansoni* chez l'enfant d'âge scolaire. Les deux tests immunologiques

sont basés sur la détection des antigènes cathodiques circulants (ACC) de *S. mansoni* dans l'urine. Troisièmement, à valider la fiabilité du test CCA-A chez les enfants d'âge préscolaire ce avant et après traitement au praziquantel. Quatrièmement, à comprendre l'épidémiologie et les facteurs de risque associés à la schistosomiase chez les enfants âge préscolaire. Cinquièmement, à évaluer l'efficacité et la sécurité du praziquantel concassé chez les enfants d'âge préscolaire dans un contexte où *S. mansoni* et *S. haematobium* co-existent.

Méthodes: Le travail de terrain pour cette thèse de doctorat a été scindé en deux parties. En 2010, notre objectif était de bien caractériser d'une part le district d'Azaguié en termes de prévalence et d'intensité des parasitoses intestinales et d'autre part d'évaluation la fiabilité des tests CCA-A et CCA-B pour le diagnostic de *S. mansoni* dans les différentes zones endémiques sélectionnées. Une étude transversale a été réalisée dans sept écoles, situées dans quatre localités du district d'Azaguié et incluant plus de 600 écoliers. Plusieurs échantillons de selles et d'urine ont été prélevés chez chacun des écoliers et cela sur trois jours consécutifs. Les échantillons de selles ont été examinés par la technique de Kato-Katz pour le diagnostic de *S. mansoni* et géohelminthiases (*Trichuris Trichiuris*, *Ascaris lumbricoides*, ankylostome). Une partie des échantillons de selles provenant de la première journée de collecte a été conservée dans une solution d'acétate de sodium acide acétique formol (SAF). Un mois plus tard, les échantillons ainsi conservés ont été examinés par la méthode de concentration d'éther pour le diagnostic des protozoaires intestinaux. Les échantillons d'urine ont été examinés avec les tests CCA (CCA-A et CCA-B) pour le diagnostic de *S. mansoni*. En outre, ces échantillons d'urine ont également été soumis à la technique de filtration d'urine et aux bandelettes réactives pour le diagnostic de *S. haematobium*.

En 2011, l'enquête de base consistait à une étude transversale mise en œuvre dans deux villages du district d'Azaguié à savoir Azaguié Makouguié et Azaguié M'Bromé où *S. mansoni* et *S. haematobium* co-existent. Environ 300 enfants d'âge préscolaire (moins de 6 ans) ont été recrutés pour cette enquête. Afin d'étudier l'épidémiologie et les facteurs de risques associés à la schistosomiase chez les enfants d'âge préscolaire et d'évaluer la fiabilité du test CCA-A pour le diagnostic de *S. mansoni* chez les préscolaires, plusieurs échantillons de selles et d'urine ont été une fois de plus collectés sur deux jours consécutifs. Les procédures de collecte et d'analyse de laboratoire des échantillons étaient similaires à celles adoptées en 2010. En plus, les mesures anthropométriques (poids, taille, circonférence du bras) et les caractéristiques cliniques (taux d'hémoglobine, température) de chaque enfant ont

été notées. Un questionnaire a été administré aux mères des enfants, y compris des discussions de groupes focaux pour l'évaluation des facteurs de risque de la schistosomiase.

Les enfants d'âge préscolaire ont été traités sur la base de leur poids, avec des tablettes de praziquantel concassées et mélangées à de l'eau. L'évaluation de l'efficacité du traitement a été réalisée trois semaines post-traitement suivant les mêmes procédures de collecte et d'analyse d'échantillons de selles et d'urine qu'à l'enquête de base. Les effets indésirables survenus chez les enfants ont été évalués en interrogeant leurs mères.

Résultats: Dans la poursuite des cinq objectifs de cette thèse de doctorat, nous avons produit des résultats qui peuvent être structurés comme suit:

Parasitoses intestinales dans le district Azaguié: Nous avons montré que la sélection des zones d'interventions par les programmes de contrôle basée sur les résultats d'analyse d'un échantillon de selles examinés avec double lames de Kato-Katz ou un échantillon d'urine analysé avec une filtration d'urine tend à sous-estimer la prévalence de la schistosomiase. Cela conduit à des erreurs de classification des zones d'intervention lorsqu'on suit les lignes directrices de l'organisation mondiale de la Santé (OMS) en matière d'intervention. D'où la nécessité d'un outil de diagnostic beaucoup plus sensible que la méthode de Kato-Katz ou d'un effort d'échantillonnage par la collecte de multiples échantillons, afin de sélectionner les zones d'intervention avec une grande fidélité. En outre, nous avons trouvé une hétérogénéité à petite échelle dans la distribution des helminthes et des protozoaires intestinaux. Nous avons également confirmé que le poly-parasitisme est commun dans le district d'Azaguié.

Fiabilité des tests CCA chez les écoliers dans différentes zones endémiques: La prévalence de *S. mansoni* dans les zones A, B et C était de 32,9%, 53,1% et 91,8%, respectivement. Dans les trois zones la sensibilité d'un seul test CCA-A était similaire à celle de triples lames Kato-Katz. La sensibilité d'un seul test CCA-A par rapport à triple lames de Kato-Katz était de 56,3% et 47,9% dans la zone A, 69,6% et 73,9% dans la zone B, et 89,6% et 94,2% dans la zone C, respectivement. La spécificité d'un seul test CCA-A était modérée (76,9 à 84,2%). La probabilité d'une intensification de la coloration de la bande du test CCA-A augmente avec un nombre d'œufs de *S. mansoni* dans les selles (odds ratio: 1,07 ; $p < 0,001$). Une infection simultanée avec *S. haematobium* ou la présence d'hématurie microscopique n'a aucune influence sur les résultats du test CCA-A dans le diagnostic de *S. mansoni*. La faible sensibilité du test CCA-B dans notre zone d'étude exclut son utilisation pour le diagnostic de *S. mansoni*.

Fiabilité du test CCA (CCA-A) chez les enfants d'âge préscolaire avant et après traitement au praziquantel: Avant le traitement, la prévalence de *S. mansoni*, tel que déterminé par quatre lames de Kato-Katz, deux tests CCA(t-) prenant en compte les "traces" comme des résultats négatifs, et deux tests CCA(t+) avec les "traces" comme des résultats positifs, était de 23,1%, 45,0% et 76,5%, respectivement. Indépendamment de la référence de diagnostic considérée, un seul test CCA (CCA(t+) ou CCA(t-)) était plus sensible comparé à quadruples lames de Kato-Katz avant et après traitement. La spécificité d'un seul test CCA était comprise entre 59,3% et 100% avant et après traitement. L'intensité de la réaction des bandes des tests CCA était positivement corrélée avec les charges d'œufs de *S. mansoni* chez les préscolaires (Odd ratio = 1,2 ; p = 0,04).

Epidémiologie de la schistosomiase chez les enfants d'âge préscolaire: La prévalence de *S. mansoni* chez les enfants d'âge préscolaire était de 21,6% à Azaguié M'Bromé et de 25,5% à Azaguié Makouguié, et celle de *S. haematobium* de 5,9% et 17,3%, respectivement. La plupart des infections étaient d'intensité légère. L'occupation des mères et des aînés des enfants d'âge préscolaire jouent un rôle important dans l'épidémiologie de la schistosomiase chez les enfants d'âge préscolaire.

Efficacité et effets indésirables du praziquantel concassé chez les enfants d'âge préscolaire: En nous basant sur la technique de Kato-Katz et la filtration d'urine, nous avons trouvé une grande efficacité du praziquantel contre *S. mansoni* (taux de guérison (TG) = 88,6%, le taux de réduction des œufs (TRE) = 96,7%) et contre *S. haematobium* (TG = 88,9%, TRE = 98,0%). Le traitement a été généralement bien toléré, mais un effet indésirable modéré (c'est-à-dire l'inflammation du corps et du visage) a été observé chez quatre enfants non infectés, ce qui a nécessité une surveillance étroite par le médecin de l'étude.

Conclusions: Des outils de diagnostic plus sensibles ou des approches d'échantillonnage rigoureuses sont nécessaires pour sélectionner les zones d'intervention avec une grande fidélité. L'hétérogénéité observée à petite échelle des infections à helminthes intestinaux et à protozoaires intestinaux devra être considérée avec soin par les programmes de lutte. Un seul test CCA est plus sensible que plusieurs lames de Kato-Katz aussi bien que chez les enfants d'âge scolaire que chez les enfants d'âge préscolaire. Le test CCA peut donc être recommandé pour l'identification rapide des communautés à haut risque. Cependant, son usage pour l'évaluation de l'impact des interventions mérite d'être approfondie. Dans notre zone d'étude, les enfants d'âge préscolaire sont à risque de la schistosomiase et peuvent être infectés très tôt

dans l'enfance. Des approches intégrées, prenant en compte, l'amélioration de l'approvisionnement en eau potable, l'assainissement, le système de santé, et l'éducation à la santé sont nécessaires au sein des communautés impliquées dans notre étude. Le praziquantel concassé est efficace contre *S. mansoni* (schistosomiase intestinale) et *S. haematobium* (schistosomiase urinaire) chez les enfants d'âge préscolaire et pourrait être recommandé pour le traitement uniquement des enfants infectés. Néanmoins, des recherches supplémentaires sont nécessaires pour approfondir notre compréhension de l'innocuité du praziquantel dans ce groupe d'âge.

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1. Introduction

1.1. Life cycle and biology of schistosomiasis

Schistosomiasis (also known as bilharziasis) is a disease caused by blood flukes of the genus *Schistosoma*. This parasitic disease, depending on the causative agent, involves either the gastrointestinal or urinary tracts of the definitive host (Gray et al., 2011). Six trematode species are known to cause schistosomiasis in humans, namely *Schistosoma haematobium*, *S. japonicum*, *S. mansoni*, *S. intercalatum* and *S. mekongi*, *S. guineensis* (Utzinger and Keiser, 2004; Gray et al., 2011; Rollinson et al., 2012). The first three species account for the majority of human disease. Unlike other trematodes which are hermaphroditic, schistosomes have male and female genders. The male is larger than the female (length: 1.2-1.6 cm, width: 0.016 cm versus length: 0.6-1.4 cm, width: 0.11 cm). The flukes have a whitish colour and a funnel-shaped oral sucker at its anterior end and a second pediculated sucker. The male has also a lamelliform shape with marginal folds forming a canal (gynaecophoric canal groove) in which the more slender female worm resides. Female schistosomes require a male to mature, and if separated from the male it will regress in maturity (Skelly, 2008). The female has a cylindrical body and is darker. During copulation the female lay copious eggs without leaving her mate; a female *S. mansoni* lays around 100-300 eggs per day, *S. haematobium* can lay up to 300 per day whereas *S. japonicum* can produce up to 3000 eggs per day (Larry et al., 2005).

Figure 1.1 shows the life cycle of *Schistosoma* spp. The life cycle is complex presenting different morphologies: the egg, the cercaria (free-swimming larval stage) and the adult worm. It involves a sexual reproduction phase in the human definitive (or final life cycle) host, an asexual phase in a snail intermediate host, and a phase in freshwater. Aquatic snails from the genus *Bulinus* act as intermediate hosts for *S. haematobium*, *S. intercalatum* and *S. guineensis*, snails from the genus *Biomphalaria* act as intermediate hosts for *S. mansoni*, *Tricula aperta* is the aquatic intermediate host for *S. mekongi* and the amphibious snail from the genus *Ocomelania* is the intermediate host for *S. japonicum* (Gryseels et al., 2006; King, 2009). Transmission occurs in bodies of freshwater or marshlands where the snail releases tiny cercariae that can penetrate the skin of the human host. Once inside the human host, the parasites develop into an immature form called schistosomula and migrate to the final location in the hepatic portal system where the two sexes pair and mature. As adults, the worm pairs of *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. guineensis* and *S. mekongi* migrate to the mesenteric venules (small blood vessels in the membranous folds of the

intestine), and the worm pairs of *S. haematobium* migrate to the vesical plexus (a network of vessels and veins that drain the ureters). At these locations, the paired worms produce eggs. Eggs that become trapped in the tissues of organs cause inflammation and severe morbidity in the chronic stages of the schistosome disease. When eggs are released into the environment (freshwater body) through the human host's excreta or urine, they hatch into miracidia, which is the ciliated larval stage that requires a snail as host in order to develop into the next stage to produce cercariae. This description of the life cycle suggests that schistosomes do not multiply in the human host and the intensity of infection in humans is largely determined by the rate at which new worms are acquired through contact with cercariae-infected water. The worms that start an infection on day 1 are the same worms present in the host days, years or even decades later. Moreover, unlike the other schistosomes, *S. japonicum* is a veritable zoonotic parasite infecting not only humans but also more than 40 mammalian species (most notably water buffalo) that constitute important reservoir hosts (Wang et al., 2005).

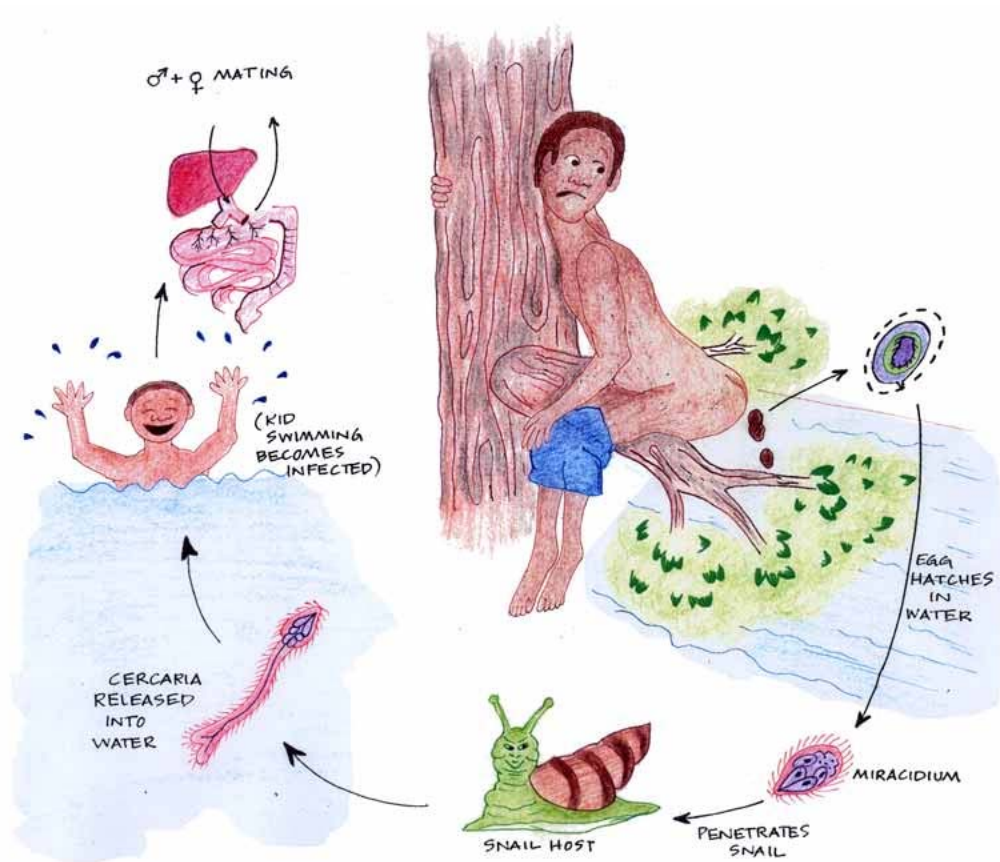


Figure 1.1: Life cycle of *Schistosoma* spp. (source: <http://parasito-nasiri.blogfa.com/post-5.aspx>)

1.2. Epidemiology

1.2.1. Geographical distribution

Schistosomiasis continues to threaten millions of people, particularly in sub-Saharan Africa (van der Werf et al., 2003; Vennervald and Dunne, 2004). Of some 779 million people exposed, an estimated 207 million people are infected, more than half of whom are symptomatic and at least 20 million exhibit severe disease manifestations (King et al., 2005; Steinmann et al., 2006). New research using Bayesian geostatistics suggests that the true number of infections is considerably higher (Schur et al., 2011; Schur et al., 2012).

Figure 1.2 shows the current distribution of schistosomiasis, which is endemic in 76 countries (Engels et al., 2002; Fenwick et al., 2006). *S. haematobium* infections are endemic in the Middle East, the African continent and some islands of the Indian Ocean (Mauritius, Madagascar, and Zanzibar), while *S. mansoni* infections are found mainly in the African continent, Madagascar, South America and the Arabian Peninsula. Schistosomiasis due to *S. japonicum* is prevalent in Southeast Asia and in Western Pacific countries. *S. intercalatum* is endemic in 10 countries in Central and West Africa, while *S. guineensis* shows an even more restricted geographical distribution. *S. mekongi* is found only on Khong Island, Lao People's Democratic Republic and in Cambodia (Utzinger and Keiser, 2004; Muth et al., 2010). With *S. japonicum* eliminated from Japan in the late 1970s, schistosomiasis in the developed world is restricted to travellers returning from endemic countries and the migration of population at risk from endemic areas (Whitty et al., 2000; Grobusch et al., 2003).

The global burden of schistosomiasis has been estimated at 1.7-4.5 million disability-adjusted life years (DALYs) (Lamine et al., 2006; Brooker et al., 2007). However, based on revised disability weights, estimates of up to 70 million DALYs have been put forth (King et al., 2005; King and Dangerfield-Cha, 2008; Hotez and Kamath, 2009).

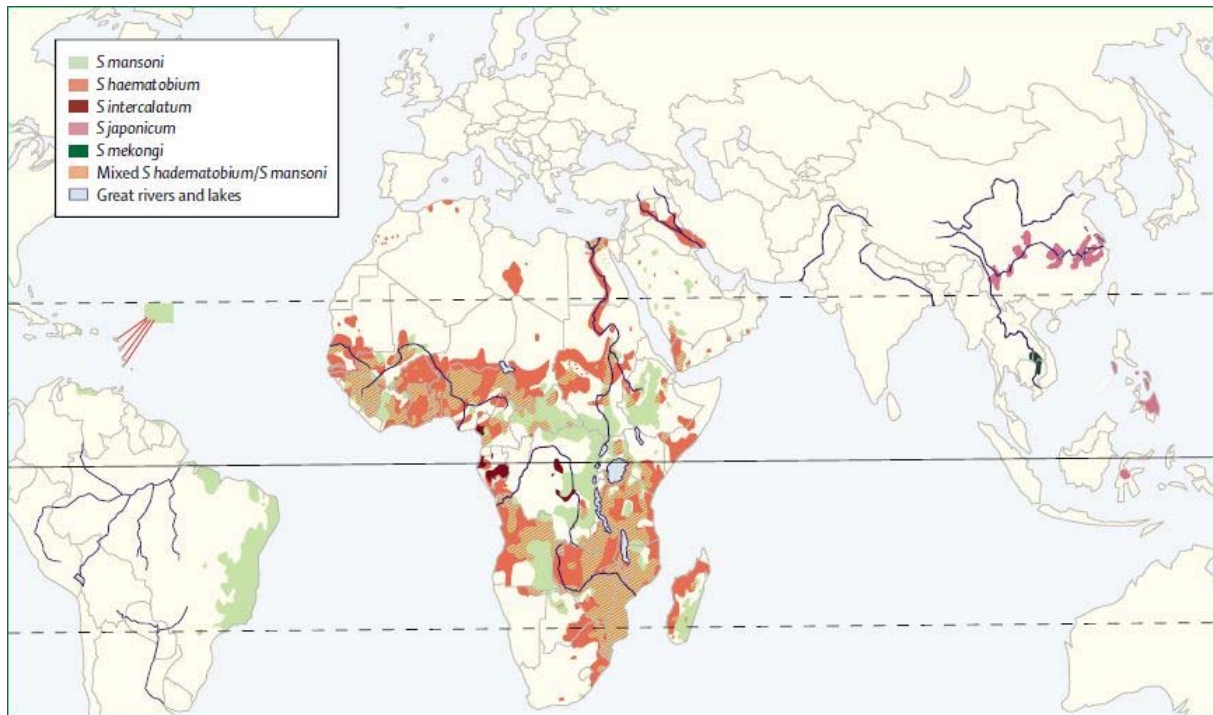


Figure 1.2: Global distribution of schistosomiasis (Gryseels et al., 2006)

1.3. Transmission

There are three main features that govern the transmission of human schistosomiasis, namely (i) focal distribution, (ii) aggregation and (iii) heterogeneity.

The geographical focality of schistosome infections is a result of the complex interrelationship between the distribution and density of infected persons and of the contaminated environment with compatible intermediate snail hosts snails, the distance between infected persons and the suitable infested freshwater bodies, which act as transmission sites, and the mean frequency, mean duration and surface of the body exposed during water contact with the infested environment (Useh and Ejezie, 1999; King, 2009). The spatial heterogeneity of schistosome infections has been demonstrated at district, country and regional levels (Raso et al., 2005; Clements et al., 2009; Schur et al., 2012) with specific foci of the infection limited to areas where all components required for disease transmission are, met simultaneously. Detailed investigations at microgeographical scale within a community are however, limited (Kloos et al., 1998).

The distribution of schistosome infection intensity within a single area is described as statistically over-dispersed. Indeed, the majority (80%) of infected people excrete only few schistosome eggs, whereas the remaining 20% excrete a large number of eggs (Guyatt et al.,

1994; Ross et al., 1998; Ellis et al., 2006). There are day-to-day and intra-specimen variations in schistosome egg output in infected individuals (Utzing et al., 2001; Booth et al., 2003). Contributing factors for variations of intensity (and for persistent re-infection) following chemotherapy might not be solely social and environmental factors. There might also be human genetic factors such as naive antibody response among children.

Immunological factors such as acquired immunity, parasite genetic variability as demonstrated by the difference in susceptibility to praziquantel (Messerli et al., 2009; Liang et al., 2011), increased innate resistance or acquired immunity to antischistosomal drugs, or a combination of these factors. Understanding risk factors associated with aggregation of worms in individual human hosts is important in understanding the transmission dynamics of schistosomiasis and for the prevention, control and eventual elimination of the infection and disease.

The distribution of compatible intermediate host snails influences the variability in the rate of schistosome infections. Key determinants for the intermediate host snail abundance are water temperature and flow velocity, which vary over time, resulting in typical seasonal transmission patterns, and they are therefore important factors in explaining the heterogeneity in time and space of the schistosomiasis epidemiological patterns (Stensgaard et al., 2012). Intermediate host snails show spatial microhabitat preferences even within a single river system (Utzing et al., 1997).

1.4. Pathology and morbidity due to schistosomiasis

Individual schistosome worms can live in the blood circulation of untreated people for many years and repeated infection and re-infection may ultimately lead to chronic disease. Investigations by van der Werf et al. (2003) found that more than half of the estimated 180 million schistosome-infected individuals in sub-Saharan Africa suffer considerable associated morbidity. Indeed, an estimated 18 million people have bladder wall pathology, 10 million have hydronephrosis and 8.5 million have hepatomegaly. These estimates highlight the public health importance of schistosomiasis and the urgent need for control not only of the primary infection but also of the associated morbidity. For children in endemic regions, persistent schistosome infection can retard growth and impair physical and cognitive function along with an array of psychological, social and long-term economic consequences (Vennervald and Dunne, 2004).

Schistosomiasis-related morbidity has two major manifestations: (i) an acute hypersensitivity reaction against migrating schistosomula known as Katayama fever (Ross et al., 2007) and (ii) chronic disease due to the presence of schistosome eggs that fail to reach the intestinal or bladder lumen and become trapped in the peri-intestinal (Andersson and Chung, 2007; Bezerra et al., 2007; Lubeya et al., 2010) or peri-vesical tissues (Brouwer et al., 2003) and the lung. Andersson and Chung (2007) reported an additional morbid state, schistosomal myelopathy, due to *S. haematobium* in Malawi.

S. haematobium affects the genito-urinary tract when eggs become trapped in the vesical or urethral walls, which causes local ulceration and pseudopolyposis manifesting as haematuria. An estimated 70 million individuals in sub-Saharan Africa have haematuria due to urogenital *S. haematobium* (Gryseels et al., 2006). Fibrotic reactions may lead to hydronephrosis and ultimately kidney failure. Bladder cancer has been linked to late-stage urogenital schistosomiasis. The public-health importance of genital schistosomiasis in both women and men (Leutscher et al., 1997; Feldmeier et al., 1999) is now being recognised as a risk factor of numerous sexually transmitted diseases, including human immunodeficiency virus (HIV) (Lawn et al., 2000; Mbabazi et al., 2011).

Morbidity in intestinal schistosomiasis caused by *S. mansoni*, *S. japonicum*, and *S. mekongi* is characterised by specific symptoms. These symptoms include anaemia, intestinal bleeding from microulcers and often bloody diarrhoea, abdominal pain, loss of appetite, vitamin A deficiency and malnutrition as well as non-specific symptoms such as nausea, tiredness and abdominal pain (Ajanga et al., 2006). Symptoms may be ill-defined and only become evident at an advanced stage of the disease. Life-threatening haematemesis from gastro-esophageal varices and pulmonary hypertension are seen in severe cases (Vennervald et al., 2004; Gryseels et al., 2006).

1.5. Morbidity assessment

To date, ultrasonography remains the ‘gold’ standard to assess morbidity in chronic organ pathology associated with schistosomiasis. This method is safe, rapid and non-invasive and invaluable for the visualization of the organ-specific schistosomiasis-associated organ change (Hatz, 2001; Richter, 2003; Richter et al., 2003). The use of ultrasonography is limited in developing countries because of its cost and the lack of well-trained examiners. Consequently, there is a pressing need for simple and inexpensive tools to document changes in morbidity as

a function of large-scale control programmes. Various methods for assessing morbidity have been used, and they include simple school-based questionnaires (Lengeler et al., 2002a; Lengeler et al., 2000b; Raso et al., 2004; Brooker et al., 2009), disease markers (Midzi et al., 2003) and clinical radiological imaging (Ortega et al., 2010). Questionnaires based on self-reported signs and symptoms have been successfully developed and widely validated for rapid and inexpensive identification of communities at high-risk for morbidity (N'Guessan N et al., 2007). However questionnaire might be subject to recall bias and lack of specificity in signs and symptoms for different helminths species (e.g. *Schistosoma*, soil-transmitted helminths, etc.).

1.6. Diagnosis of schistosomiasis

1.6.1. Parasitological diagnosis

1.6.1.1. Kato-Katz and urine filtration methods

Direct detection of schistosome eggs in urine (i.e. *S. haematobium*) and stool samples (e.g. *S. mansoni* and *S. japonicum*) under a microscope is the most widely used diagnostic approach in epidemiological surveys of schistosomiasis. A commonly employed direct method for the diagnosis of urogenital schistosomiasis is the standard urine filtration method that involves the detection and quantification of *S. haematobium* eggs in a 10-ml filtrate of a mid-day urine specimen (Plouvier et al., 1975; Mott et al., 1982). The Kato–Katz technique for quantification of faecal egg counts, originally developed in the mid-1950s by the Japanese researchers Kato and Miura (Kato and Miura, 1954) and further modified in the early 1970s by Katz and colleagues in Brazil (Katz et al., 1972), is the most widely used technique in epidemiological surveys pertaining to intestinal schistosomiasis (and is also widely used for soil-transmitted helminth infections). This technique is simple, but requires a minimum of laboratory equipment (e.g. microscope and mostly reusable test kit materials) and well-trained laboratory technicians. Most commonly, Kato–Katz thick smears are prepared by using 41.7 mg plastic templates (Speich et al., 2010).

In an early stage of a control programme, when morbidity control is the declared objective, infection prevalence and intensity are usually high, and hence direct methods show reasonable accuracy. However, when prevalence, and particularly intensity of infection are reduced through treatment, direct methods become less sensitive and should be augmented or replaced by immunological techniques based on antigen or antibody detections (van Lieshout

et al., 2000; Bergquist et al., 2009; Johansen et al., 2010), or molecular tools such as polymerase chain reaction (PCR)-based approaches (Gomes et al., 2010). It is widely acknowledged that single Kato–Katz thick smear examinations underestimate the ‘true’ prevalence of *S. mansoni* and *S. japonicum* and this issue is particularly important in settings where infection intensities are low (Utzinger et al., 2001; Booth et al., 2003; Yu et al., 2007; Lin et al., 2008). Numerous studies have investigated the effect of stool consistency, intra-specimen and day-to-day variation on faecal egg output, and have discussed the implications for research and control (Utzinger et al., 2001; Booth et al., 2003; Enk et al., 2008). Based on this body of research, multiple stool samples with at least duplicate Kato–Katz thick smears per sample should be analyzed to have a reasonable sensitivity. Another approach to improve diagnostic sensitivity is to employ multiple methods for the same stool sample, e.g. the Kato–Katz technique combined with the ether-concentration method or the FLOTAC method (Raso et al., 2006; Legesse and Erko, 2007; Steinmann et al., 2008; Knopp et al., 2008).

1.6.1.2. Immunodiagnosis

Immunodiagnosis of schistosomiasis has been proposed to overcome some of the limitations with parasitological methods that is day-to-day and intra-specimen variation of schistosome egg output, the risk of missing low-intensity infections, relatively time-consuming methodologies and the need for well-trained laboratory technicians (van Lieshout et al., 2000; Doenhoff et al., 2004).

Immunological approaches are based on the detection of antibodies or antigens in urine or blood samples. Although immunodiagnosis usually requires somewhat better equipped laboratories and much more knowledge than direct techniques using microscopy, immunological methods, may yield higher sensitivity, especially for antibody detection. However, specificity might be a problem for antibody detection, and since antibody detection is not quantitative, it is difficult to differentiate between light and heavy infections. Moreover, antibody levels remain high for prolonged periods of time following treatment, which represents a diagnostic dilemma: failure to differentiate between active and cured infections. Finally, there might be a high degree of cross-reactivity in settings where schistosome and other trematode infections co-exist (Bergquist et al., 2009; Johansen et al., 2010). In the People’s Republic of China, a common approach for the diagnosis of *S. japonicum* is to first screen at-risk populations for antibodies in blood, followed by stool microscopy of antibody-positive individuals (Utzinger et al., 2005; Zhu, 2005; Balen et al., 2007). In Venezuela, the

combination of stool microscopy with serological methods has been proposed for the diagnosis of schistosomiasis mansoni in low-transmission areas (Alarcón de Noya et al., 2007).

Detection of schistosome antigens, such as circulating anodic antigens (CAA) and circulating cathodic antigens (CCA) (van Lieshout et al., 2000) or *S. mansoni* soluble egg antigen (SEA) (Chand et al., 2010) in blood or urine, using enzyme-linked immunosorbent assays (ELISAs) hold several advantages over antibody detection. Most notably, active infections can be readily demonstrated. Hence, this approach is useful for anthelmintic drug efficacy trials due to high specificity. Classical ELISA procedures, however, are quite slow, require well-equipped laboratories and highly qualified technicians (van Lieshout et al., 2000). Against this background, different rapid diagnostic assays have been developed. A promising test is based on the detection of CCA in urine for the diagnosis of schistosomiasis. The principle of the test is based on a lateral-flow assay using a nitrocellulose strip of the sample with a colloidal carbon conjugate of anti-CCA monoclonal antibodies (van Dam et al., 2004). However, additional validation of the CCA urine strip test for the diagnosis of both urogenital and intestinal schistosomiasis in different epidemiological settings is warranted. A rapid diagnostic test based on urine examination, characterized by high sensitivity and specificity would be a major asset, particularly for the diagnosis of *S. mansoni* at point-of-care (POC). This should render this technique a useful diagnostic approach at POC in resource-constrained settings where schistosomiasis is entrenched (Stothard et al., 2009b). Figure 1.3 shows a commercially available POC-CCA cassette test with positive, negative or invalid test results.

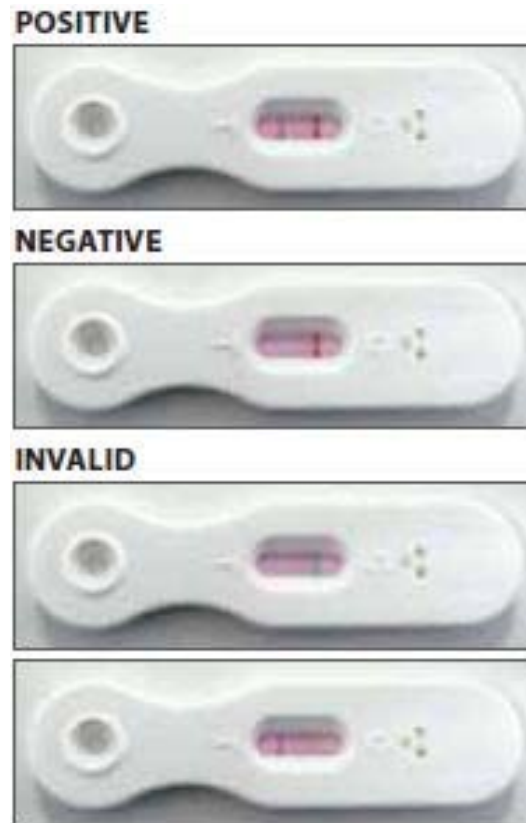


Figure 1.3: Interpretation of POC-CCA cassette results (www.rapid-diagnostics.com)

1.6.1.3. Molecular diagnosis

Diagnostic tools with a high sensitivity are required for the detection of light-infection intensities. Additionally, such tools are needed for the rigorous evaluation of drug efficacy trials and monitoring of late-stage schistosomiasis control programmes, when transmission control and local elimination become the ultimate targets. In 2002, the proof-of-concept PCR for the detection of *S. mansoni* in faecal samples was published by a group in Brazil (Pontes et al., 2002). In the meantime, several other groups have developed additional specific and highly sensitive PCR-based assays for schistosomiasis diagnosis (Gomes et al., 2010).

The method is based on the amplification of a highly repeated DNA sequence, using simple DNA extraction techniques and a rapid two-step PCR approach, which facilitated the amplification of *S. mansoni* DNA in faecal samples. Recently, a multiplex real-time (RT)-PCR for the detection and quantification of both *S. mansoni* and *S. haematobium* has been developed (ten Hove et al., 2008). Research in Ghana, placing emphasis on the diagnosis of *S. haematobium*, confirmed the high sensitivity and specificity of RT-PCR, compared to standard urine filtration and CCA strip tests (Obeng et al., 2008). Given the high sensitivity of PCR-based methods, operational advantages (need for only a single stool or urine sample),

the potential for high throughput and the possibility for extension to other helminths or intestinal protozoa using on additional molecular targets, this approach provides a powerful diagnostic platform for epidemiological research and might become the ‘gold’ standard during the end game of helminth control programmes.

1.6.1.4. Metabolic profiling

A diagnostic approach detecting potential biomarkers, discovery in the *S. mansoni*-mouse model and based on a metabolic profiling strategy has been presented (Wang et al., 2004). This approach allows assessing and quantifying the dynamics of biochemical responses of living systems to patho-physiological stimuli (Nicholson et al., 1999).

Metabolic profiling is useful for studying an organism’s gene function, drug safety assessment and recovery of biomarkers (Lindon et al., 2004; Holmes, 2010). Progress made thus far with metabolic profiling investigations to enhance our understanding of patho-physiological responses has been reviewed (Wang et al., 2010). In brief, metabolic profiling provides a new platform for biomarker discovery and might lead to the generation of novel diagnostic assays. However, this diagnostic approach will require considerable financial and technical resources for knowledge and technology transfer to resource constrained countries where schistosomiasis is endemic (Holmes, 2010; Wang et al., 2010).

1.6.1.5. Clinical diagnosis

Other diagnostic alternatives include a asking for clinical signs and symptoms examining rectal biopsies, among others.

The use of questionnaires for schistosomiasis screening has been evaluated quite extensively in sub-Saharan Africa (Lengeler et al., 2002a; Lengeler et al., 2002b; Brooker et al., 2009). While the use of questionnaires generally is perceived as useful, even though not without problems, for *S. haematobium* screening, the diagnostic performance for *S. mansoni* is much weaker and the usefulness thus more debated (Lengeler et al., 2002; Lengeler et al., 2002b). Especially those people with light infections might lack any specific symptoms and signs related to schistosomiasis. The classical signs and symptoms (e.g. abdominal pains, colicky cramps, blood in stool, diarrhoea and hepatomegaly or splenomegaly are not pathognomonic or characteristic only for intestinal schistosomiasis. The utility of questionnaires in low prevalence areas must be validated locally (Lengeler et al., 2002; Lengeler et al., 2002). The main advantages of simple school-based questionnaires are their

low costs, the non-need of invasive diagnostic procedures and the collaboration between the health and education sectors.

Schistosomiasis can be diagnosed by examining a biopsy from the rectal mucosa. The biopsy is crushed between two glass slides and examined under a microscope directly or after adding a few drops of glycerol-malachite solution. By this method the viability of the eggs can be assessed. However, due to the invasive nature of the technique, it is usually limited to assessment of selected patients in a hospital or large clinical setting and not used on a routine basis or in screening programmes. The utility of the technique must be weighed against the alternative of performing repetitive stool microscopy tests or a combination of different diagnostic tests (Rabello, 1992). Figure 1.4 shows steps for diagnostic tests development, barriers and proposed solutions to overcome mentioned barriers.

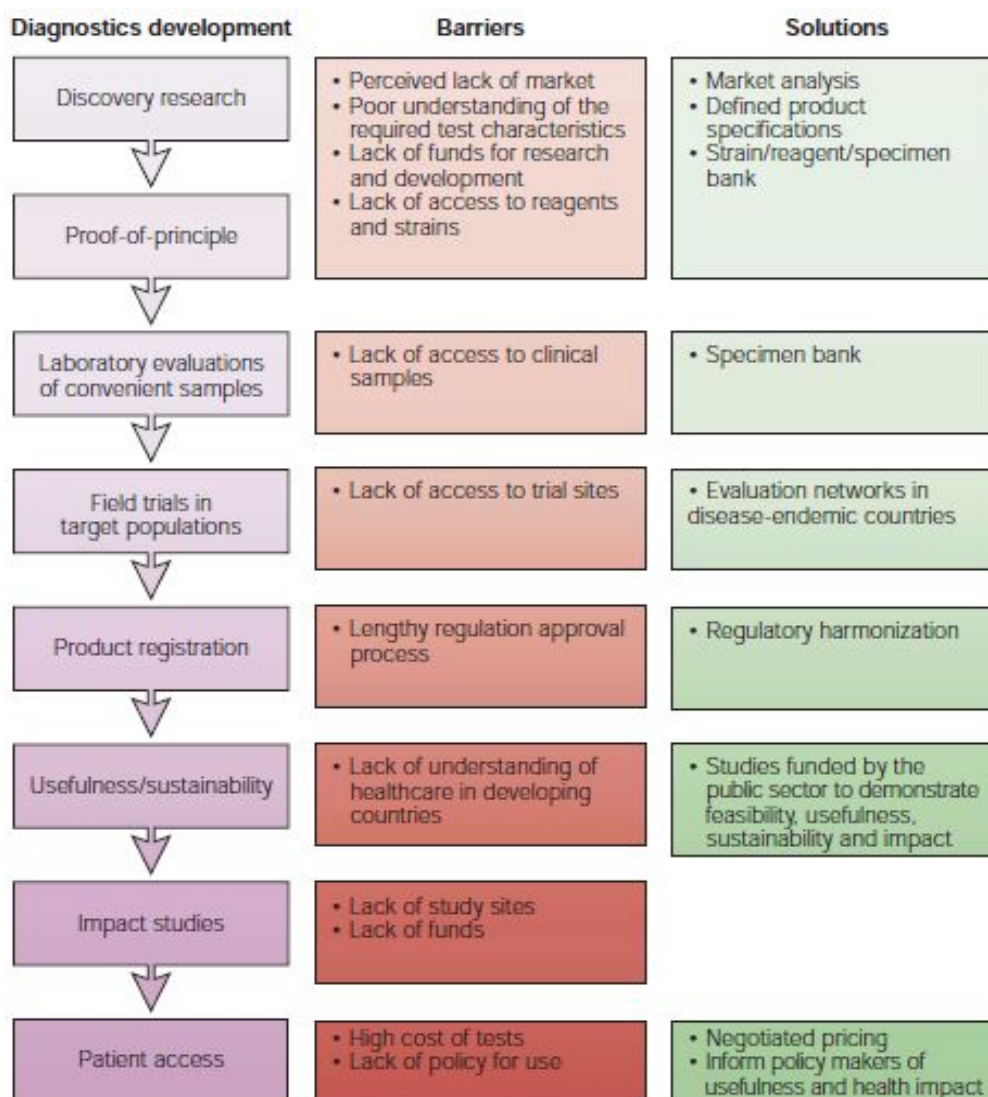


Figure 1.4: Diagnostics development: steps, barriers and solution (Mabey et al., 2004)

1.7. Control strategies of schistosomiasis

1.7.1. Treatment of schistosomiasis

The drug of choice against schistosomiasis is praziquantel (WHO, 2002; Fenwick et al., 2003; Utzinger and Keiser, 2004; Doenhoff et al., 2008). Praziquantel is usually administered in a single oral dose of 40 mg/kg of body weight. For, large-scale community-based control programmes, praziquantel is administered on a simple, widely validated ‘dose pole’ (Montresor et al., 2001; Montresor et al., 2002). The use of this dose pole facilitated dosing based on people’s height rather than weight, thus without the need for balances, which are usually not available in resource-constraint settings. Praziquantel is efficacious against all six human schistosome species and has a good safety profile. Oxaminiquine (Thiong'o et al., 2002) has been widely used for the treatment of schistosomiasis mansoni, particularly in Brazil (Katz, 2008), usually producing cure rate >70%. However, oxaminiquine is not active against schistosome species other than *S. mansoni* (Fenwick and Webster, 2006). Metrifronate is active against *S. haematobium* but no longer on the WHO model list of essential drugs (Feldmeier and Chitsulo, 1999; Danso-Appiah et al., 2008). Artemisinins (e.g., artesunate and artemether) show activity against juvenile schistosome parasites (Xiao et al., 2002; Utzinger et al., 2007). Other drugs such as amodiaquine and sulphadoxine-pyrimethamine (SP) were also investigated for their efficacy against schistosomes. Recently, the antimalarial drug mefloquine was described to have *in vitro* and *in vivo* activity against *S. mansoni* (Keiser et al., 2009). First proof-of-concept trials showed interesting results, particularly when mefloquine was combined with other drugs (Keiser et al., 2010). Some researchers have shown that drug combinations (e.g. praziquantel + artemether, SP + artemether, amodiaquine + artemether) might produce higher cure rates than a single drug regimen. The aforementioned drugs are generally well tolerated (Keiser and Utzinger, 2007; Utzinger et al., 2007).

Most anthelmintic drugs have had a substantial price reduction over the past several years. For example, treatment of a schistosomiasis patient is now in the order of US\$ 0.30 (Fenwick et al., 2003; Doenhoff et al., 2008). However these drugs provide limited choice as none of the compounds has a large parasite spectrum and they were mostly developed decades ago (Utzinger et al., 2007). Indeed, there is an urgent need to rapidly develop safe and effective new drugs to add to the existing treatment option for schistosomiasis. It is encouraging to note that new efforts are getting under way to evaluate alternative

anthelmintic drugs or drug combinations of existing drugs (Bethony et al., 2006; Keiser and Utzinger, 2007).

1.7.2. Control strategies

Chemotherapy-based interventions for the rapid reduction of infection-related morbidity and community prevalences remain the current mainstay for the control of schistosomiasis and other helminthiasis (Savioli et al., 2005; Fenwick et al., 2009; Hotez et al., 2010). In May 2001, the 54th World Health Assembly (WHA) recommended (in WHA resolution 54.19) the widespread use of preventive chemotherapy as the central feature for the control of schistosomiasis and soil-transmitted helminthiasis. The stated objective was to regularly treat, by the year 2010, at least 75%, and up to 100%, of all school-aged children at risk of schistosomiasis and soil-transmitted helminthiasis (WHO, 2002; Fenwick et al., 2003). Thus, WHA resolution 54.19 led the way for concurrently controlling multiple neglected tropical diseases (Savioli et al., 2009; Utzinger et al., 2009).

Summarized in Table 1.1 are WHO guidelines for the community treatment of helminthiasis. If the prevalence exceeds 50%, entire communities should receive treatment; if the prevalence is between 10% and 50%, only school-aged children receive treatment; if the prevalence is below 10%, school-aged children might be treated at least twice, at school entry and again before they finish schooling.

Table 1.1: Recommended treatment strategies by WHO for schistosome infections.

Community category	Prevalence in school survey	Intervention in schools
I High prevalence	≥30% visible haematuria (<i>S. h.</i> by questionnaire) or ≥50% infected (<i>S. m.</i> and <i>S. h.</i> , by parasitological methods)	Targeted treatment of school-aged children, once a year
II Moderate prevalence	<30% (<i>S. h.</i> by questionnaire) or ≥10% but <50% infected (<i>S. m.</i> and <i>S. h.</i> , by parasitological methods)	Targeted treatment of school-aged children, once every 2 years
III Low prevalence	<10% infected (<i>S. m.</i> and <i>S. h.</i> , by parasitological methods)	Targeted treatment of school-aged children twice during primary schooling (once on entry, again on leaving)

S.m., *S. mansoni*; *S. h.*, *S. haematobium*

In line with WHA resolution 54.19, various large-scale preventive chemotherapy programmes for the rapid reduction of morbidity and community prevalences due to neglected

tropical diseases have been launched in numerous countries of Africa and elsewhere (Kabatereine et al., 2006; Hotez et al., 2007; Fenwick et al., 2009; WHO, 2011; WHO, 2012). Although the evaluation of their true effects is challenging (Brooker et al., 2004), these programmes are usually assumed to be highly effective (Molyneux, 2004). However, some limitations of these preventive chemotherapy programmes are worth to be highlighted. First, 40% of children in sub-Saharan Africa may not be enrolled in school. Yet, the coverage is an important factor determining the effectiveness of control programmes emphasizing preventive chemotherapy (Rollinson et al., 2012). Second, issues of integration and sustainability have received insufficient attention (Utzinger et al., 2009). Without additional actions to improve water supply and sanitation, enhanced food safety, health education, general improvements to living conditions, the benefits of preventive chemotherapy programmes focused on school-aged children are probably not sustainable in the long term (Asaolu and Ofoezie, 2003; Utzinger et al., 2003; Rollinson et al., 2012). Sound health education campaigns coupled with community participation can achieve a change of human behaviour by increasing awareness in the population about the transmission mode and health consequences of helminthiasis (Useh and Ejezie, 1999; Rollinson et al., 2012). Focal mollusciciding for schistosomiasis transmission control in specific geographical settings is still important today but it has high costs and is of limited duration and effectiveness, as snails re-populate their habitats shortly after interventions (Chu, 1976).

Integrating helminth control programmes and linking them with efforts to hold back other issues such as malnutrition, tuberculosis, malaria and HIV/AIDS have been advocated repeatedly (Molyneux and Nantulya, 2005; Brady et al., 2006) and estimates suggest considerable cost savings (Brady et al., 2006). Current efforts to control schistosomiasis are inadequate and a new generation of tools is needed for disease control, along with appropriate environmental control measures, health education as a target of control programmes (Stothard et al., 2009a; Utzinger et al., 2009). One such new generation tool which holds the best prospect for the sustainable control of schistosomiasis is the development of vaccines that will prevent the parasite from completing its life cycle (Bethony et al., 2011; Hotez et al., 2011).

1.8. Schistosomiasis in preschool-aged children

1.8.1. Paucity of literature

“It’s an old explorer’s adage that you only find what you are looking for and that, without considerable pre-planning, ‘discoveries’ are not always as serendipitous as they first seem” (Stothard et al., 2011). Although, schistosomiasis has been found long time ago in preschool-aged children it received only in past decade the attention of scientific community (Smith, 1958; Perel et al., 1985). In addition, schistosomiasis was noted in 1996, to be the possible cause of death of Brazilian preschool-aged children after autopsy (Gryseels and De Vlas, 1996). This was due to a largely disjointed literature which was a collection of sporadic reports failing to synergise (Woolhouse et al., 2000). In absence of sensitive diagnosis methods to detect infected preschool-aged children and their unlike contact with freshwater it became widely believed that infections were very rare in this younger age-class (Jordan and Webbe, 1969). Furthermore, this apparent lack of exposure became an argument, sufficient to result in categorisation of preschool-aged children as largely free from water-linked disease (Jordan and Webbe, 1969). During this period, school-aged children and adolescents were the main target of strategies to combat schistosomiasis. This has been emphasized by the resolution 54.19 which stated that, in order to achieve millennium development goal (MDG) number 6, it was necessary to treat at least 75% and up to 100% of school-aged children in schistosomiasis endemic areas (WHO, 2002).

1.8.2. Towards awareness

The realisation that schistosome infection and disease were occurring in preschool-aged children started to take form during discussions in London in May 2003, during the first annual Schistosomiasis Control Initiative (SCI) review meeting. It has been shown during this meeting by film that poor sanitation and water-hygiene conditions abounded in several SCI-supported countries (Beanland et al., 2006). It was clearly shown that infants and preschool-aged children were being regularly bathed with freshly drawn environmental water, either at the water’s source or at home, which highlighted the fact that preschool-aged children are much more exposed to freshwater than previously thought. This was confirmed by high levels of disease prevalence in school-aged children attending reception class (between 5–7 years old) in Ugandan primary schools (Kabatereine et al., 2007). This situation was also confirmed by several epidemiological surveys focusing on preschool-aged children (<6 years) in Ghana and Uganda (Bosompem et al., 2004; Odogwu et al., 2006). Moreover, these infections were

most likely not acquired by active water contact of the child but rather by passive exposure(s) to water containing schistosome cercariae owing to the bathing and water-drawing practices of their mothers/guardians. This was clearly affirmed by Odogwu et al. (2006) who interviewed mothers of the children with a semi-structured water contact questionnaire.

1.8.3. *A gap in praziquantel treatment*

The current drug of choice for schistosomiasis, praziquantel was first released under a patent by Bayer (Leverkusen, Germany) in 1979 and, at that time, praziquantel underwent the mandatory toxicology testing. However, despite few data to suggest a potentially adverse outcome, this drug was never studied on pregnant or lactating women and on infants (Allen et al., 2002). Stothard and Gabrielli in (2007) were the first authors of the provocative attempt to highlight the importance of schistosomiasis in the younger child and this ‘praziquantel treatment gap’, set within the context of preventive chemotherapy. They reported on the present inequality of health care provision and outlined some steps that needed to be undertaken to move towards inclusion of younger children within disease control strategies. Foremost was to foster inter-sectoral collaborations with other stakeholders committed to maternal and child health. The article stimulated some debate and points raised by Johansen and colleagues (2007) were formally discussed by in a reply by Stothard and Gabrielli (2007a). They conclude that preschool-aged children inclusion in treatment campaigns should follow further thematic research necessary to optimise interventions and ensure safety (Johansen et al., 2007; Stothard and Gabrielli, 2007a). There have been several other reports investigating the occurrence of schistosomiasis in younger children across sub-Saharan Africa (Mafiana et al., 2003; Opara et al., 2007; Sousa-Figueiredo et al., 2008; Chu et al., 2010; Ekpo et al., 2010; Garba et al., 2010; Dabo et al., 2011; Namwanje et al., 2011; Verani et al., 2011). Garba et al. in (2010) documenting that *S. haematobium* and *S. mansoni* co-infections are common among very young children in Niger. It important to note that children respond to each parasite separately, or in combination through time is not well-understood. Recently, several investigations proposed: First, an extension of the praziquantel ‘dose pole’. As these shoreline environments are typical resource-poor settings, the availability of reliable weighing scales was constrained and a limiting factor in a public health setting (Sousa-Figueiredo et al., 2010). Therefore, a praziquantel ‘dose pole’ was needed similar to that used in treatment of school-aged children (Montresor et al., 2001; Montresor et al., 2002). Second, preschool-aged children are currently treated with crushed praziquantel tablets (Keiser et al., 2011). However,

due to the taste and adverse events of this treatment regimen, a new formulation of praziquantel (Garba et al., 2010; Olliaro et al., 2011).

1.9. Goal and specific objectives

1.9.1. Goal

The overarching goal of this Ph.D. thesis was to deepen our understanding of the epidemiology of schistosomiasis in preschool-aged children in a highly endemic area of Côte d'Ivoire. This called for identification of the most suitable study area and characterization of helminth and intestinal protozoa infections among children in the district of Azaguié, validation of alternative diagnostic test for *S. mansoni*, placing emphasis on urine CCA, efficacy and safety of praziquantel (using crushed tablets) in preschool-aged children.

1.9.2. Specific objectives

The present Ph.D. thesis pursued 5 specific objectives:

- To characterize Azaguié district in term of prevalence and intensity of helminths and intestinal protozoa infections.
- To assess a POC urine-based CCA test as a diagnostic tool for *S. mansoni* in school and preschool-aged children in Azaguié, south Côte d'Ivoire.
- To assess a POC urine-based CCA test as a diagnostic tool for *S. mansoni* in preschool-aged children before and after crushed praziquantel administration in Azaguié, south Côte d'Ivoire.
- To assess the epidemiology and risk factors of schistosomiasis in infants and preschool-aged children in Azaguié, south Côte d'Ivoire.
- To assess the efficacy and adverse events of praziquantel in infants and preschool-aged children in Azaguié, south Côte d'Ivoire.

1.10. References

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2. Intestinal parasitic infections in schoolchildren in different settings of Côte d’Ivoire: effect of diagnostic approach and implications for control

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2.1. Abstract

Background: Social-ecological systems govern parasitic infections in humans. Within the frame of assessing the accuracy of a rapid diagnostic test for *Schistosoma mansoni* in Côte d'Ivoire, three different endemicity settings had to be identified and schoolchildren's intestinal parasitic infection status was characterized.

Methods: In September 2010, a rapid screening was conducted in 11 schools in Azaguié district, south Côte d'Ivoire. In each school, 25 children were examined for *S. mansoni* and *S. haematobium*. Based on predefined schistosome endemicity levels, three settings were selected, where schoolchildren aged 8-12 years were asked to provide three stool and three urine samples for in-depth examination of parasitic infections. Triplicate Kato-Katz thick smears were prepared from each stool sample for *S. mansoni* and soil-transmitted helminth diagnosis whereas urine samples were subjected to a filtration method for *S. haematobium* diagnosis. Additionally, a formol-ether concentration method was used on one stool sample for the diagnosis of helminths and intestinal protozoa. Multivariable logistic regression models were employed to analyze associations between schoolchildren's parasitic infections, age, sex and study setting.

Results: The prevalences of *S. mansoni* and *S. haematobium* infections in the initial screening ranged from nil to 88% and from nil to 56%, respectively. While in the three selected areas, the rapid screening revealed prevalences of *S. mansoni* of 16%, 33% and 78%. Based on the more rigorous diagnostic approach the respective prevalences increased to 92%, 53% and 33%. *S. haematobium* prevalences were 0.8%, 4% and 65% (rapid screening results: 0.0%, 0.0% and 54%). Prevalence and intensity of *Schistosoma* spp., soil-transmitted helminths and intestinal protozoa infections showed setting-specific patterns. Infections with two or more species concurrently, were most common in the rural setting (84%), followed by the peri-urban (28.3%) and urban setting (18.2%).

Conclusions: More sensitive diagnostic tools or rigorous sampling approaches are needed to select endemicity settings with high fidelity. The observed small-scale heterogeneity of helminths and intestinal protozoa infections has important implications for control.

2.2. Background

Intestinal parasitic infections (e.g. helminths and pathogenic intestinal protozoa) are of considerable public health importance, particularly in developing countries. For example, the global burden caused by soil-transmitted helminthiasis (infections with *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) is estimated at 39 million disability-adjusted life years (DALYs), whereas the burden due to schistosomiasis (mainly *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*) is estimated at 4.5 million DALYs (WHO, 2002; Hotez et al., 2006). Amoebiasis due to infections with the intestinal protozoon *Entamoeba histolytica* results in 40,000-100,000 deaths each year (Stanley, 2003), and giardiasis due to *Giardia intestinalis* might affect 200 million people per annum (Minenoa and Avery, 2003). However, the burden of pathogenic intestinal protozoan infections in terms of DALYs remains to be determined, which is a challenge due to the paucity of up-to-date epidemiological data (Hotez et al., 2009).

Parasitic infections are governed by behavioural, biological, environmental, socioeconomic and health systems factors. Local conditions, including access and quality of domestic and village infrastructure, economic factors such as disposable income, employment and occupation, and social factors such as education, influence the risk of infection, disease transmission and associated morbidity and mortality (Yakubu et al., 2003; Wang et al., 2009; Aagaard-Hansen et al., 2009).

As a preparatory step for a rigorous assessment of the accuracy of a new rapid diagnostic test for *S. mansoni* in Côte d'Ivoire, we aimed to identify a low prevalence (10-24%) and a moderate prevalence (25-49%) of *S. mansoni* endemicity setting, and a third setting where *S. mansoni* and *S. haematobium* co-exist. While in the initial screening only single stool and urine samples were collected from each individual. In the main study, the diagnosis was based on multiple stool and urine examinations to more accurately characterize schoolchildren's intestinal parasitic infection profiles in the selected study areas. Implications of our findings for the design, implementation and monitoring of intestinal parasite control strategies in Côte d'Ivoire are discussed.

2.3. Methods

2.3.1. Ethical considerations

This study was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland) and received clearance from the ethics committees in Basel (EKBB, reference no. 377/09) and Côte d'Ivoire (reference no. 1993 MSHP/CNER). The local authorities (i.e. village chiefs, school directors, teachers and medical staff) were informed about the objectives and procedures of the study. Literate parents and legal guardians of eligible schoolchildren were given an information sheet, whereas those who were illiterate were informed in lay terms by the teachers. Written informed consent was obtained from parents/guardians, whereas children assented orally. Participation was voluntary, and hence, children could withdraw from the study at any time. At the end of the study, all participating schools were offered free treatment regardless of the infection status of the children. Praziquantel manufactured by Bayer (single 40 mg/kg oral dose using a dose-pole) and albendazole obtained from GlaxoSmithKline (single 400 mg oral dose) were administered by medical staff.

2.3.2. Selection of study settings

The study was conducted within the frame of a multi-country project funded by the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) to assess the accuracy of a rapid diagnostic urine circulating cathodic antigen (CCA) test for *S. mansoni* infections in different endemicity settings (Coulibaly et al., 2011). For the current investigation in Côte d'Ivoire, three *S. mansoni* endemicity settings were to be identified, as follows: (i) low *S. mansoni* endemicity setting (i.e. prevalence of 10-24%); (ii) moderate *S. mansoni* endemicity setting (i.e. prevalence of 25-49%); and (iii) setting where *S. mansoni* and *S. haematobium* are co-endemic. Recent research conducted in the district of Azaguié revealed a variety of schistosome endemicity scenarios (Ouattara et al., 2010; N'Guessan et al., 2006; Glinz et al., 2010), and hence this district, located approximately 40 km north of Abidjan (Figure 5), was chosen for the current investigation. In September 2010, we carried out a rapid screening in 11 schools. Twenty-five children, aged 8-12 years, were randomly selected by drawing lots in each school. Children provided one stool and one urine sample that were examined with standard techniques.

Based on the screening results, three schools that fitted in the prevalence scheme indicated above were selected. All children aged 8-12 years were considered eligible for

participation. According to sample size calculations for the overarching SCORE project, around 200 children from existing school lists were randomly selected in each of the three settings and invited to participate in the main study.

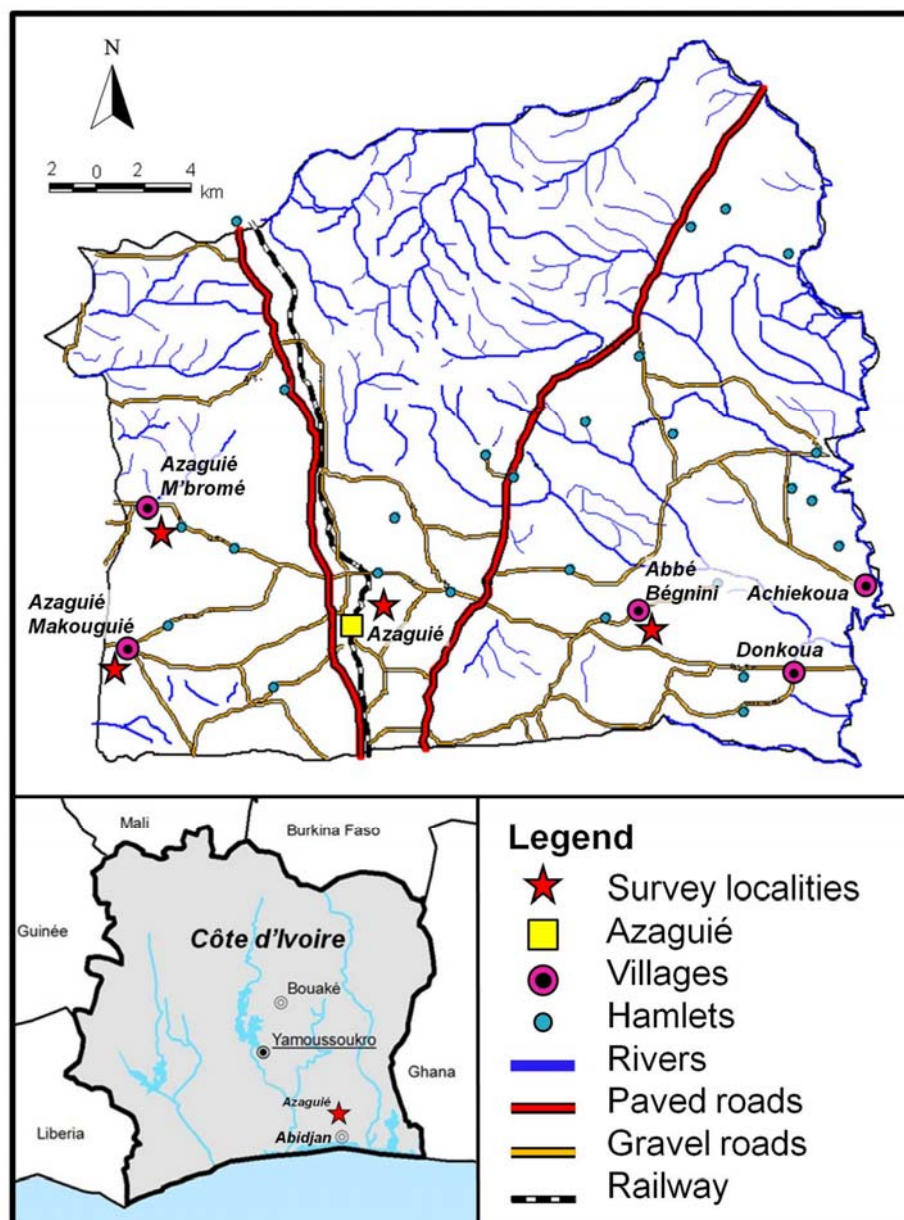


Figure 2.1: Map showing the district of Azaguié in south Côte d'Ivoire. Indicated are Azaguié town and its surrounding villages. Among the 11 schools included in the pre-screening, five were located in the surrounding villages and six were located in the Azaguié town area. The three settings selected for the in-depth studies (i.e. Azaguié M'Bromé/Azaguié Makouguié, rural; Abbé-Bégnini, peri-urban; Azaguié Gare, urban) are emphasised with red stars.

2.3.3. *Field procedures*

For the main study, in the early morning, right after school lessons had started, children were given plastic containers labelled with unique identification numbers (IDs) and invited to return the containers filled with a fresh morning stool sample (10-50 g) the following morning. Upon sample collection, new empty containers were handed out for stool collection the next morning. This procedure was repeated over three days. Catch-up collections were done for two more days, and by then most of the children had submitted three stool samples. Additionally, after the children had provided stool samples, they were given another container and asked to provide a urine sample to be produced between 10:00 and 12:00 hours.

2.3.4. *Laboratory procedures*

Stool and urine collections were completed around noon. The samples were transferred to a parasitological laboratory at the Université de Cocody in Abidjan and processed the same day as follows. First, triplicate 41.7 mg Kato-Katz thick smears were prepared from each stool sample (Katz et al., 1972). The slides were allowed to clear for at least 30 min before examination under a microscope by one of five experienced laboratory technicians. The number of helminth eggs were counted and recorded for each species separately. Second, from each participant's second day stool sample, ~1 g of stool was placed into a Falcon tube containing 10 ml of sodium acetate-acetic acid-formalin (SAF) solution, broken and homogenized with a wooden spatula and vigorously shaken. Third, urine samples were shaken and 10 ml filtered using plastic syringe and filter-holders with 13-mm diameter filter (mesh size 20 µm) (Sefar AG; Heiden, Switzerland). Filters were removed with forceps, placed on microscope slides, a drop of Lugol's solution added and examined under a microscope. The number of *S. haematobium* eggs was counted and recorded.

Within 6-10 weeks of stool collection, the SAF-fixed samples were subjected to an ether-concentration method, using a standard protocol (Utzinger et al., 2008). In brief, the SAF-fixed stool samples were re-suspended and filtered through a medical gauze placed in a plastic funnel into a centrifuge tube. The tube was centrifuged for 1 min at 500 g. After centrifugation, the supernatant was discarded and 7 ml of 0.85% NaCl plus 2-3 ml of ether were added to the remaining pellet. After shaking for 10-30 s, the tube and its content were centrifuged for 5 min at 500 g. Finally, from the four layers formed, the three top layers were discarded. The bottom layer, including the sediment, was examined under a microscope. The number of helminth eggs were counted and recorded for each species separately. Intestinal

protozoa were recorded semi-quantitatively as follow: (i) negative (no cysts or trophozoites in the entire sediment); (ii) light (one to five cysts or throphozoites per slide); (iii) moderate (one cyst or trophozoite per observation field at a magnification of x 400 or x 500); and (iv) heavy (more than one cyst or trophozoite per observation field at a magnification of x 400 or x 500) (Utzinger et al., 2008).

2.3.5. *Statistical analysis*

Data were double entered and cross-checked using EpiInfo version 3.2 (Centers for Disease Control and Prevention; Atlanta, GA, USA). Statistical analyses were carried out using STATA version 10 (Stata Corporation; College Station, TX, USA).

Parasite species-specific data analysis was restricted to those children who had complete data records (i.e. three stool samples examined with triplicate Kato-Katz thick smears for *S. mansoni* and soil-transmitted helminths, one stool sample subjected to an ether-concentration method for helminths and intestinal protozoa and three urine samples examined with a single urine filtration for *S. haematobium*).

For each individual, arithmetic mean egg counts of the helminths were calculated from the nine Kato-Katz thick smears and three urine filtration readings. Helminth infection intensities were expressed as eggs per gram of stool (EPG) and eggs/10 ml of urine (for *S. haematobium*) and classified into light, moderate or heavy, according to thresholds put forth by the World Health Organization (WHO) (WHO, 1998). Helminth infection intensities were also estimated at the setting level, using the group's arithmetic mean faecal egg counts.

Dichotomous data are presented as proportion in the pre-screening as well as for the three main study settings. For the latter settings, multivariable logistic regression models were fitted for each parasite. The prevalence of a respective parasite was used as dependent variable (binary variable: present/absent) and the prevalence of each other parasite, sex (binary variable: male/female), age (continuous variable) and the study settings (categorical variable) were used as explanatory variables. As preparatory analysis for assessment of our multivariable logistic regressions, in each setting, univariable logistic regression was used to assess the association between each dependent variable (parasite infection) and each covariate (i.e. sex, age, village and other parasites). Subsequently, interactions between sex, age and village were assessed for each dependent variable. Next, village effects were assessed by considering a clustered structure in the models (xtlogit command in STATA). That was, we considered a random intercept for each village in the logistic regression model. Village effects

for the respective parasites were fitted in each final model. At the end, a backward stepwise elimination procedure was applied, including interactions and village effect to determine significant association between parasitic infections and between parasitic infections and study setting. The explanatory variable with the highest p-value in the multivariable logistic regression model was eliminated before re-running the multivariable logistic regression model and these iterations of elimination were continued as long as the values of the Akaike information criterion (AIC) of the new models were decreasing. A similar approach has already been successfully used in other studies (Raso et al., 2004). For all statistical results, a p-value below 0.05 was considered as statistically significant.

2.4. Results

2.4.1. Prescreening

Table 2.1 shows the results of the initial screening carried out in 11 schools in Azaguié district. According to triplicate Kato-Katz thick smears derived from a single stool sample obtained from 25 children per school, the prevalence of *S. mansoni* ranged between nil and 88%. Abbé-Begnini with a *S. mansoni* prevalence of 16% was the only low endemicity school, whereas the three schools in Azaguié Gare were determined as moderately endemic as the *S. mansoni* prevalences were between 25% and 49%. *S. haematobium* infections, determined by single urine filtrations, were found in seven of the 11 schools. In five of these schools, only one to three children were infected. However, in Azaguié M'Bromé and Azaguié Makouguié, more than half of the children were infected with *S. haematobium*.

Hence, the settings Abbé-Begnini, Azaguié Gare and Azaguié M'Bromé/Azaguié Makouguié were selected as they fitted SCORE's predefined classifications of low, moderate and mixed endemicity, respectively.

Table 2.1: Prevalence of *S. mansoni* and *S. haematobium*, as assessed in an initial screening carried out in 11 schools in Azaguié district, south Côte d'Ivoire in September 2010.

School	No. (%) of infected children		Endemicity ^a
	<i>S. mansoni</i>	<i>S. haematobium</i>	
Abbé-Begnini	4 (16)	0	Low
Achiékoua	0	0	Not selected
Ahoua 1	14 (56)	1 (4)	Not selected
Ahoua 2	14 (56)	1 (4)	Not selected
Ahoua 3	15 (60)	2 (8)	Not selected
Azaguié Gare 1A	9 (36)	0	Moderate
Azaguié Gare 2A	7 (28)	0	Moderate
Azaguié Gare 2B	9 (36)	3 (12)	Moderate
Bambou	11 (44)	1 (4)	Not selected
Azaguié M'Bromé	17 (68)	14 (56)	Mixed
Azaguié Makouguié	22 (88)	13 (52)	Mixed

^a Endemicity was set according to SCORE guidelines: prevalence of *S. mansoni* between 10% and 24% indicates low endemicity, prevalence of *S. mansoni* between 25% and 49% was considered moderate endemicity, co-existence of *S. mansoni* and *S. haematobium* indicates mixed endemicity.

In each school, the prevalence was assessed among 25 randomly selected children, aged 8-12 years. One stool sample was examined with triplicate Kato-Katz thick smears to determine the prevalence of *S. mansoni*, whereas one urine sample was subjected to a single filtration to assess the prevalence of *S. haematobium*.

2.4.2. *Characteristics of study settings and population*

Adhering to SCORE guidelines, Abbé-Begnini (low endemicity *S. mansoni* setting), Azaguié Gare (moderate endemicity *S. mansoni* setting) and Azaguié M'Bromé/Azaguié Makouguié (mixed *S. mansoni*-*S. haematobium* setting) were selected for an in-depth appraisal of school-aged children's helminths and intestinal protozoan infection profiles. Interestingly, Azaguié M'Bromé/Azaguié Makouguié, Abbé-Begnini and Azaguié Gare (Figure 2.1) were located in rural, peri-urban and urban areas, respectively. The proportions of children with complete data records from the sampling of stool and urine were 77.3% in Azaguié M'Bromé/Azaguié Makouguié, 62.4% in Abbé-Begnini and 59.1% in Azaguié Gare.

Azaguié M'Bromé and Azaguié Makouguié, two small villages located approximately 9 and 12 km west of Azaguié town, are typical rural settings. These two villages are difficult to access via gravel roads that are infrequently used by private transport. People are mainly engaged in subsistence farming. There is no tap water supply and households lack sanitation facilities, and hence the population practices open defecation. In each village, there is a primary school. Additionally, in Azaguié M'Bromé, there is a primary health care centre.

Abbé-Begnini is a peri-urban village located approximately 5 km east of the center part of Azaguié town. The village is reachable on a tarmac road by private and public transport. Subsistence farming is the main source of income. Sanitation coverage is low and inhabitants depend on open surface water from the nearby river for household use and on two water pumps for drinking and cooking. There is one primary health care centre.

Azaguié Gare is an urban neighbourhood of Azaguié town, which has excellent transport connections to Abidjan (train, bus and car). The population mainly consists of civil servants, artisans, traders and some farmers. Most of the inhabitants have access to tap water and only few use public man-made wells. Latrines with cemented slabs or flush toilets are common. The town has several private primary health care centres and a public secondary health facility.

2.4.3. *Intestinal parasitic infections*

Figure 2.2 shows the study adherence. Overall, 170, 146 and 130 schoolchildren had complete parasitological data in Azaguié M'Bromé/Azaguié Makouguié, in Abbé-Begnini and in Azaguié Gare, respectively. Table 2.2 shows the infection prevalence of intestinal parasites in the main study, stratified by setting. In rural Azaguié M'Bromé/Azaguié Makouguié, very high prevalences of both *S. mansoni* and *S. haematobium* were found (91.8% and 65.3%,

respectively). *T. trichiura* is the predominant soil-transmitted helminth (56.5%). While hookworm is also common (44.7%), the prevalence of *A. lumbricoides* is considerably lower (11.2%). *Entamoeba coli* with a prevalence of 31.8% was found to be the predominant intestinal protozoon infection, followed by *Endolimax nana* (28.8%), *Blastocystis hominis* (15.9%) and *G. intestinalis* (8.8%).

In peri-urban Abbé-Begnini, hookworm and *S. mansoni* showed the highest prevalences, 41.1% and 32.9%, respectively. The prevalence of *S. haematobium*, *T. trichiura* and *A. lumbricoides* were all below 10%. *E. coli* and *E. nana* were the predominant intestinal protozoa with respective prevalences of 26.7% and 24.7%.

In the urban Azaguié Gare, more than half (53.1%) of the children harboured *S. mansoni*. The prevalences of hookworm, *T. trichiura* and *A. lumbricoides* were 20.8%, 15.4% and 13.1%, respectively. Only one child (0.8%) was found with *S. haematobium* eggs in the urine. In this setting, *E. nana* was the predominant intestinal protozoan infection (42.3%), followed by *E. coli* (25.4%) and *B. hominis* (10.2%).

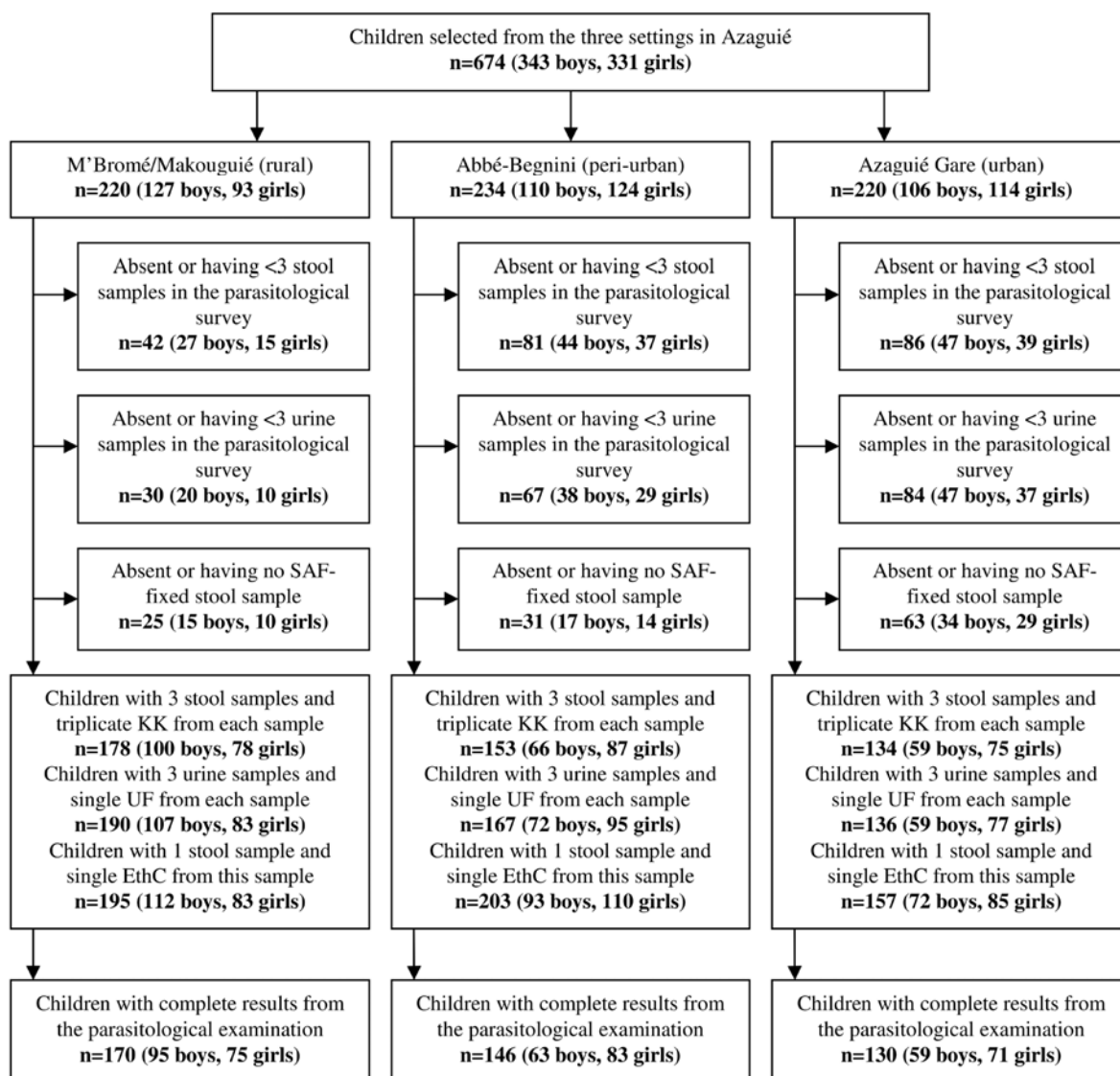


Figure 2.2: Flow chart detailing the study participation and compliance. Children were selected from three different settings in Azaguié district, south Côte d'Ivoire in October and November 2010. KK, Kato-Katz method; UF, urine filtration method; EthC, ether-concentration method.

Table 2.2: Prevalence of helminths and intestinal protozoa infections in three settings of Azaguié district, south Côte d’Ivoire in October and November 2010.

Intestinal parasite	Study setting					
	Azaguié M'Bromé/Azaguié					
	Makouguié (rural)		Abbé-Begnini (peri-urban)		Azaguié Gare (urban)	
	No. of infected	% (95% CI)	No. of infected	% (95% CI)	No. of infected	% (95% CI)
Helminths						
<i>Schistosoma mansoni</i>	156	91.8 (87.6-95.9)	48	32.9 (25.2-40.6)	69	53.1 (44.4-61.8)
<i>Schistosoma haematobium</i>	111	65.3 (58.1-72.5)	6	4.1 (0.1-7.4)	1	0.8 (0-2.0)
<i>Trichuris trichiura</i>	96	56.5 (48.9-63.9)	10	6.9 (2.7-10.9)	20	15.4(9.1-21.7)
Hookworm	76	44.7 (37.2-52.3)	60	41.1 (33.0-49.2)	27	20.8 (13.7- 27.8)
<i>Ascaris lumbricoides</i>	19	11.2 (6.4-15.9)	10	6.9 (2.7-10.9)	17	13.1 (7.2-18.9)
Intestinal protozoa						
<i>Entamoeba coli</i>	54	31.8 (24.7-38.8)	39	26.7 (19.5-33.9)	33	25.4 (17.8-32.9)
<i>Endolimax nana</i>	49	28.8 (21.9-35.7)	36	24.7 (17.6-31.7)	55	42.3 (33.7-50.9)
<i>Blastocystis hominis</i>	27	33.0 (16.9-41.7)	14	9.6 (4.8-14.4)	14	10.8 (5.4-16.2)
<i>Giardia lamblia</i>	15	8.8 (4.5-13.1)	11	7.5 (3.2-11.9)	12	9.2 (4.2-12.3)
<i>Chilomastix mesnili</i>	14	8.2 (4.1-12.4)	7	4.8 (1.3-8.3)	6	4.6 (0.9-8.3)
<i>Entamoeba histolytica/E. dispar</i>	14	8.2 (4.1-12.4)	12	8.2 (3.7-12.7)	7	5.4 (1.5-9.3)
<i>Iodamoeba bütschlii</i>	9	5.3 (1.9-8.7)	8	5.5 (1.7-9.2)	10	7.7 (3.1-12.3)
<i>Entamoeba hartmanni</i>	5	2.9 (0.4-5.5)	8	5.5 (1.7-9.2)	9	6.9 (2.5-11.3)

n = 170 (rural setting), n = 146 (peri-urban setting), n = 130 (urban setting)

Diagnosis of *S. mansoni* and soil-transmitted helminths were based on nine Kato-Katz thick smears (three stool samples, each subjected to triplicate Kato-Katz). Diagnosis of *S. haematobium* was based on three urine samples, each subjected to a single filtration. Diagnosis of intestinal protozoa was based on a single stool sample fixed in SAF that was examined with an ether-concentration technique.

2.4.4. *Infection intensities*

Infection intensities, expressed as group arithmetic mean faecal egg counts, showed some heterogeneity between settings (Table 2.3). Infection intensity classes for helminth and intestinal protozoa, stratified by setting, are summarised in Table 2.4. In rural Azaguié M'Bromé/Azaguié Makouguié, more than two-thirds of *S. mansoni*-infected children had either a moderate (35.3%) or a heavy (41.7%) infection. *S. haematobium*, hookworm, *T. trichiura* and *A. lumbricoides* infections were mainly of light intensities. Most of the children were heavily infected with intestinal protozoa. In peri-urban Abbé-Begnini, 97.0% of the infected schoolchildren showed light helminth infection intensities. Intestinal protozoa infection intensities showed no clear intensity patterns. In urban Azaguié Gare, all helminth infections were of light intensities. Intestinal protozoa infections were light (58.4%) or moderate (36.5%) and only 13 children showed a heavy infection.

Table 2.3: Geometric mean helminth eggs per gram of stool (EPG), stratified by setting.

	Azaguié M'Bromé/Azaguié Makouguié (rural)		Abbé-Begnini (peri- urban)		Azaguié Gare (urban)	
	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI
<i>S. mansoni</i>	158.1	115.6-216.1	1.1	0.7-1.6	62.0	36.0-88.0
<i>S. haematobium</i>	4.1	2.9-5.5	0.1	0.0-0.2	0	0.0-0.1
<i>A. lumbricoides</i>	0.4	0.2-0.6	0.5	0.2-0.9	0.8	0.3-1.4
<i>T. trichiura</i>	9.9	6.5-14.8	0.2	0.1-0.4	0.6	0.3-0.9
Hookworm	3.6	2.4-5.1	3.9	2.5-5.9	0.7	0.4-1.2

CI, confidence interval

Table 2.4: Categories of helminths and intestinal protozoan infection intensities, stratified by setting. Helminths infection intensities were categorized according to the classification of WHO (WHO, 1998) and intestinal protozoan infection intensities were classified as described elsewhere (Utzinger et al., 2008).

Parasite	Study setting								
	M’Bromé and Makouguié (rural)			Abbé-Begnini (peri-urban)			Azaguié Gare (urban)		
	Light (%)	Moderate (%)	Heavy (%)	Light (%)	Moderate (%)	Heavy (%)	Light (%)	Moderate (%)	Heavy (%)
Helminths									
<i>Schistosoma mansoni</i>	36 (23.1)	55 (35.3)	65 (41.7)	46 (95.8)	1 (2.1)	1 (2.1)	47 (68.1)	17 (24.6)	5 (7.2)
<i>Schistosoma haematobium</i> ^a	91 (81.9)		20 (18.0)	5 (83.3)		1 (16.7)	0		1 (100)
Hookworm	75 (100)	0	0	59 (98.3)	1 (1.7)	0	27 (100)	0	0
<i>Trichuris trichiura</i>	95 (95.0)	5 (5.0)	0	10 (90.9)	1 (9.1)	0	20 (100)	0	0
<i>Ascaris lumbricoides</i>	19 (100)	0	0	10 (100)	0	0	16 (94.1)	1 (5.9)	0
Intestinal protozoa									
<i>Entamoeba coli</i>	11 (20.4)	18 (33.3)	25 (46.3)	7 (17.9)	14 (35.9)	18 (46.2)	15 (45.5)	12 (36.4)	3 (9.1)
<i>Endolimax nana</i>	14 (28.6)	9 (18.4)	26 (53.1)	7 (19.4)	13 (36.1)	17 (47.2)	26 (47.3)	23 (41.8)	7 (12.7)
<i>Blastocystis hominis</i>	8 (29.6)	5 (18.5)	11 (40.7)	7 (50.0)	3 (21.4)	1 (7.1)	9 (64.3)	5 (35.7)	0
<i>Giardia intestinalis</i>	1 (6.7)	4 (26.7)	10 (66.7)	1 (9.1)	3 (27.3)	7 (63.6)	8 (66.7)	3 (25.0)	1 (8.3)
<i>Chilomastix mesnili</i>	2 (14.3)	4 (28.6)	8 (57.1)	1 (14.3)	1 (14.3)	5 (71.4)	3 (50.0)	2 (33.3)	1 (16.7)
<i>Entamoeba histolytica/E. dispar</i>	4 (28.6)	8 (57.1)	2 (14.3)	3 (25.0)	4 (33.3)	5 (41.7)	6 (85.7)	2 (28.6)	1 (14.3)
<i>Iodamoeba bütschlii</i>	0	3 (33.3)	6 (66.7)	4 (50.0)	1 (12.5)	3 (33.3)	8 (80.0)	2 (25.0)	0
<i>Entamoeba hartmanni</i>	1 (20.0)	1 (20.0)	3 (60.0)	4 (50.0)	2 (25.0)	2 (25.0)	8 (88.9)	1 (11.1)	0

a Infection intensity cut-off for *S. haematobium* are 1-49 eggs per 10 ml of urine (light) and ≥ 50 eggs per 10 ml of urine (heavy).

The study was carried out in Azaguié, south Côte d'Ivoire in October and November 2010. *S. mansoni* and soil-transmitted helminth infection intensities were based on the reading of nine Kato-Katz tick smears. Intestinal protozoa infection intensities were based on one SAF-fixed stool sample subjected to an ether-concentration technique. Three urine filtrations were done to determine infection intensities of *S. haematobium*.

2.4.5. *Multiparasitism*

Figure 2.3 shows the patterns of multiple species intestinal parasite infections, stratified by setting. In the rural setting, the prevalence of schoolchildren with a single, dual or multiple species helminth infection was 9.2%, 22.4% and 61.6%, respectively. In the peri-urban setting, 42.1%, 22.1% and 6.2% of the children harboured one, two or at least three helminth species concurrently. In the urban setting, single helminth species infections were most common (41.2%), whereas dual species helminth infections were found in 18.2% of the children investigated (Figure 3A).

Multiple species intestinal protozoan infections were assessed with a single ether-concentration test. In rural Azaguié M'Bromé/Azaguié Makouguié, 29.9% of the children harboured single species intestinal protozoan infections, whereas 21.3% harboured two species of intestinal protozoa concurrently. An infection with three or more intestinal protozoa species was found in 12.5% of the children. In peri-urban Abbé-Begnini, 36.5%, 29.3% and 5.1% showed single, dual or multiple species intestinal protozoan infections. In urban Azaguié Gare, 40.1% harboured single species intestinal protozoan infections, whereas dual or multiple species infections were found in 21.6% and 5.4%, respectively (Figure 3B).

Considering all intestinal parasite species (i.e. helminths and intestinal protozoa), the prevalence of single, dual or multiple species infections in the rural setting was 2.9%, 15.2% and 79.5%, respectively. Two schoolchildren harboured eight or 10 intestinal parasites concurrently. In the peri-urban setting, 39.4%, 32.4% and 28.2% of the schoolchildren harboured single, dual and multiple species infections, respectively. In the urban setting, the prevalence of single, dual and multiple intestinal parasite infections was 23.0%, 35.7% and 33.3%, respectively (Figure 3C).

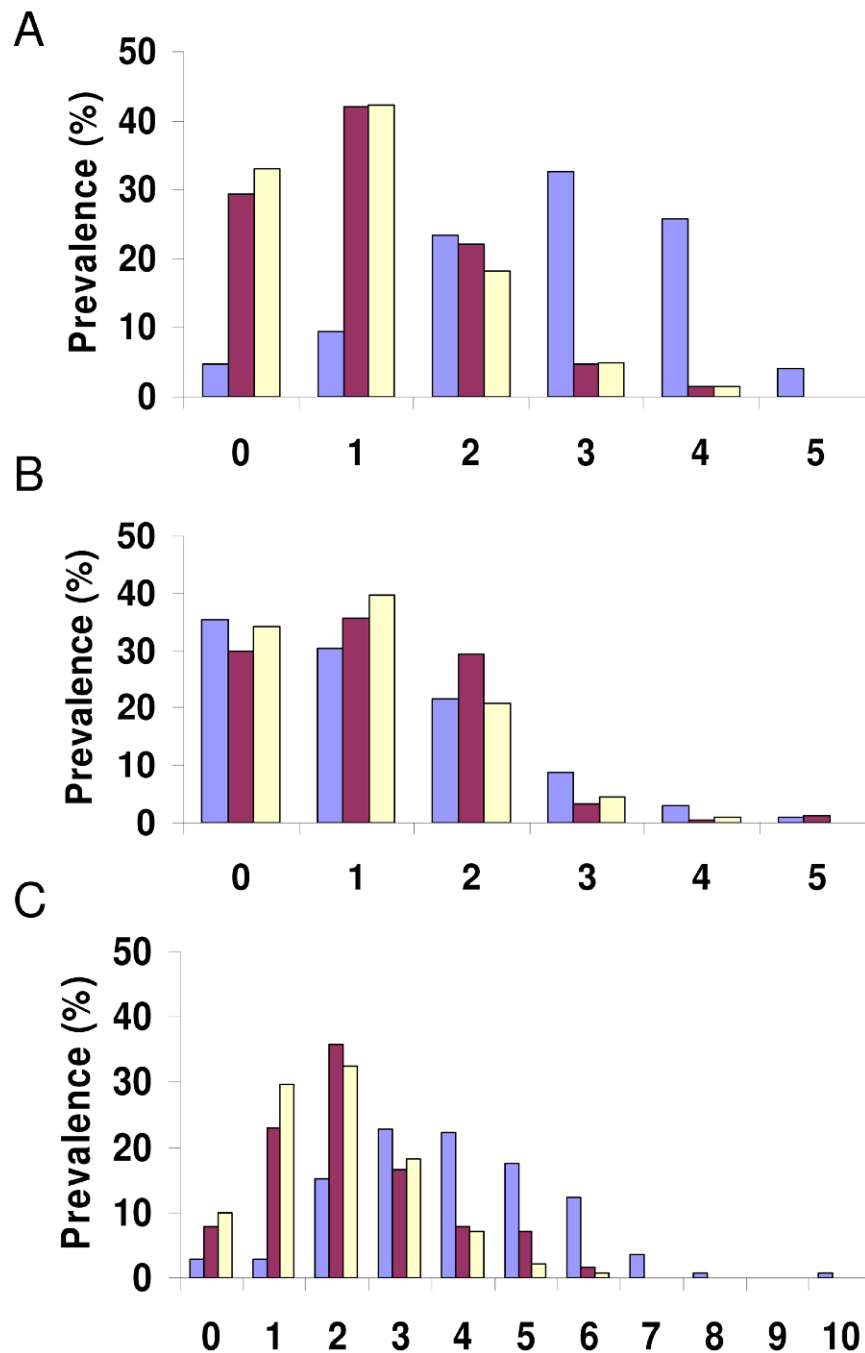


Figure 2.3: Multiple intestinal parasitic infections among schoolchildren aged 8-12 years in three settings of Azaguié district, south Côte d'Ivoire, in October and November 2010. Blue bars indicate rural Azaguié M'Bromé/Azaguié Makouguié; purple bars indicate peri-urban Abbé-Begnini; and yellow bars indicate urban Azaguié Gare. (A) Number of helminth species diagnosed per child; (B) number of intestinal protozoa species diagnosed per child; (C) number of intestinal parasites (helminths and intestinal protozoa) diagnosed per child.

2.4.6. Parasite associations

All significant associations ($p < 0.05$) between intestinal parasites, sex, age and setting resulting from the multivariable logistic regression models are summarised in Table 2.5. *S. mansoni* infection showed significant positive associations with *S. haematobium* (odds ratio (OR) = 4.81, $p = 0.005$) and *T. trichiura* (OR = 2.74, $p = 0.005$). The odds of being infected with *S. mansoni* increased with age (OR = 1.55, $p < 0.001$). *A. lumbricoides* was significantly associated with *T. trichiura* (OR = 4.24, $p < 0.001$) and hookworm (OR = 2.24, $p < 0.020$). Boys were more likely to be infected with hookworm than girls (OR = 1.87, $p = 0.003$). *E. coli* was significantly associated with *S. haematobium* (OR = 1.73, $p = 0.019$).

Table 2.5: Significant associations of intestinal parasitic infections among schoolchildren in Azaguié district, south Côte d’Ivoire, in October and November 2010.

Parasite	Association	Adjusted OR (95% CI)	p-value
Schistosomiasis			
<i>Schistosoma mansoni</i>	<i>S. haematobium</i>	4.81 (1.79-12.93)	0.005
	<i>Trichuris trichiura</i>	2.74 (1.34-5.60)	0.005
	Age	1.55 (1.29-1.87)	< 0.001
<i>Schistosoma haematobium</i>	<i>S. mansoni</i>	4.09 (1.65-10.84)	0.005
Soil-transmitted helminths			
<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	4.24 (1.96-9.19)	< 0.001
	Hookworm	2.34 (1.14-4.41)	0.02
	Rural setting	0.31 (0.13-0.73)	0.007
<i>Trichuris trichiura</i>	<i>S. mansoni</i>	2.89 (1.42-5.91)	0.003
	<i>A. lumbricoides</i>	4.14 (1.90-8.99)	< 0.001
Hookworm	<i>A. lumbricoides</i>	3.03 (1.56-5.87)	0.001
	sex	1.87 (1.24-2.81)	0.003
Intestinal protozoa			
<i>Endolimax nana</i>	<i>Blastocystis hominis</i>	2.39 (1.33-4.29)	0.004
	Peri-urban setting	0.44 (0.26-0.74)	0.002
	Rural setting	0.50 (0.31-0.82)	0.006
<i>Entamoeba coli</i>	<i>S. haematobium</i>	1.73 (1.09-2.72)	0.019
<i>Blastocystis hominis</i>	<i>Endolimax nana</i>	2.44 (1.37-4.35)	0.003

n = 446

CI, confidence interval; OR, odds ratio

Associations between a particular parasite (as binary variable; reference, absence) as dependent variable and age (as continuous variable), sex (as binary variable; reference, female), study setting (as categorical variable; reference, urban setting) and any of the remaining parasites (as binary variable, reference, absence) were analysed with multivariable logistic regression models, performing a stepwise backward elimination procedure.

2.5. Discussion

We studied patterns of intestinal parasite infections (i.e. schistosomes, soil-transmitted helminths and intestinal protozoa) in school-aged children in three settings of Côte d'Ivoire; a rural, peri-urban and urban area. An important aspect of parasitic disease control programme is the ability to readily identify and reach people at highest risk of infection and associated morbidity. Often, the poorest people are the least accessible ones living in remote rural areas, and hence they are at highest risk of parasitic infection and other conditions of ill-health (Asaolu and Ofoezie, 2003; Huang and Manderson, 2005, Hotez, 2008).

In this study, the assessment of children's prevalence and intensity of *S. mansoni* and soil-transmitted helminth infections was based on nine Kato-Katz thick smears derived from three stool samples. The day-to-day and intra-specimen variation in helminth egg output that compromise the sensitivity of the Kato-Katz method, especially in areas of low infection intensity, is overcome by such a rigorous diagnostic approach (Utzinger et al., 2001; Knopp et al., 2008; Enk et al., 2008). However, multiple stool sampling to increase the sensitivity of the Kato-Katz technique for helminth diagnosis might result in reduced compliance. Indeed, particularly in the urban setting, compliance for providing all three stool samples was considerably lower than in the rural and peri-urban settings and this might have introduced some bias.

Another aspect of our study worth highlighting is the following. After our pre-screening based on a single stool sample, and then employing a more rigorous diagnostic approach, the previously anticipated *S. mansoni* endemicity levels were considerably overrun. As predicted by mathematical modelling (de Vlas and Gryseels, 1992) and confirmed in field studies, repeated stool sampling with multiple Kato-Katz thick smears prepared from individual stool samples, result in considerable increases of the observed prevalence of *S. mansoni* (Utzinger et al., 2001; Booth et al., 2003; Enk et al., 2008). The same observations have also been made for soil-transmitted helminths (Knopp et al., 2008; Steinmann et al., 2008). It follows that if rapid screenings are performed to reliably select a study setting or treatment scheme according to prevalence estimates, more sensitive diagnostic tools as alternative to a rigorous sampling approach (i.e. collecting multiple stool samples and examining them with multiple Kato-Katz thick smears) are needed, particularly in areas with low transmission rates.

The current study confirms that schoolchildren from a rural setting are at higher risk of helminth infections than their counterparts living in peri-urban or urban settings. The remoteness of the rural setting, characterized by the absence of key infrastructures (e.g.

tarmac road, health facilities, tap water and basic sanitation) play important roles. Our observations are in line with previous epidemiological surveys; indeed, unsafe hygiene, water and sanitation and inadequate management of the environment exacerbate parasite infections in general, and helminth infections in particular (Wang et al., 2009; King, 2010; Huang and Manderson, 2005; Hotez, 2008; Ziegelbauer et al., 2012). Greatest differences between the prevalence of helminth infections, as a function of the study setting, were found with regard to the two schistosome species. Besides socioeconomic risk factors, the transmission of *S. mansoni* and *S. haematobium* is governed by intermediate host snails (Yakubu N. et al., 2003; Aagaard-Hansen et al., 2009; Stensgaard et al., 2012), and hence the availability of suitable snail habitats seem to vary considerably between the three settings even at this small-scale. While it is commonly believed that schistosomiasis is a “rural disease”, some studies have shown high prevalence in urban settings (Matthys et al., 2007). Hence, detailed malacological investigations are needed to deepen our understanding of the epidemiology of schistosomiasis with regard to the level of urbanization. Interestingly, similar hookworm prevalences were found in the rural and peri-urban settings. This observation could be explained by the fact that the behaviour of the schoolchildren with regard to hygiene and faecal management in particular is similar. Since open defecation is widely practiced in these communities, efforts must be made to improve sanitation, which in turn will have major ramifications on other neglected tropical diseases such as amoebiasis and giardiasis (Keiser et al., 2002b; Ziegelbauer et al., 2012).

Interestingly, the prevalence of intestinal protozoa infections among schoolchildren was found to be similar in the three settings. This might indicate that hygiene related to food consumption among schoolchildren is similar and needs to be improved. The two predominant intestinal protozoa species in the three settings under investigation are *E. coli* and *E. nana*. This observation is in agreement with previous studies done in different parts of Côte d'Ivoire (Keiser et al., 2002b; Ouattara et al., 2010; Traoré et al., 2011).

Regarding intestinal parasite infection intensities, with the exception of *S. mansoni* in the rural setting, all other helminth infections were of light intensities whatever the setting. The observed moderate and heavy *S. mansoni* infections in the rural setting might be explained by high transmission in the absence of preventive and curative measures. Indeed, we are not aware of large-scale prior deworming activities in the district of Azaguié. There was a tendency of infection intensities of intestinal protozoa to decrease from rural to urban

settings. Socioeconomic factors and educational attainment increase from rural to urban settings, and hence might explain this decrease in the intensities of intestinal protozoa.

Our study also confirms that multiparasitism is pervasive as observed elsewhere in sub-Saharan Africa and Asia (Brooker et al., 2000; Chungue et al., 1995; Keiser et al., 2002a; Raso et al., 2004; Steinmann et al., 2010). We found two children in the rural setting harbouring eight or 10 intestinal parasites concurrently. This high number of parasite species in a single host is an alarming situation, as multiple species infection may increase susceptibility to other parasites (Druilhe et al., 2005; Mwangi et al., 2006; Nacher, 2004). Associations between different parasite species, as well as the influence of age, sex and study setting, have been assessed in the current investigation. Of particular note is the strong positive association between *S. mansoni* and *S. haematobium* with an adjusted OR of around four. Moreover, we found a significant association between *S. haematobium* and *E. coli*, which has not been described in the literature before. Boys and girls were at the same level of exposure to helminths and intestinal protozoa, excepted for hookworm where boys were more exposed than girls. Interestingly, a previous study carried out by Keiser et al. (2002) in western Côte d’Ivoire found that hookworm infections were significantly more often diagnosed in girls (Keiser et al., 2002a; Raso et al., 2004). Behavioural and socioeconomic factors might explain this observed difference. Our study is in line with previous investigations that the risk to be infected with *S. mansoni* increases with age in children (Hotez et al., 2008; Tchuem Tchuenté, 2012). To date, the effects of parasite interactions on the human body remain poorly understood. Without a deeper understanding of such parasite interactions, the effectiveness of parasitic disease control programmes are compromised (Lello et al., 2012)

2.6. Conclusion

Continued and concerted efforts should be made by control programmes to reach rural school-aged children and other high-risk groups in the most remote areas, as they are the least accessed ones whose health and wellbeing is insufficiently accounted for by policy makers. Improvement of safe water supply and sanitation facilities by the construction of toilets could significantly reduce the burden of parasitic diseases in the rural and peri-urban settings studied here. In addition, particular importance might be given to health education at the district level. Control programmes should carefully consider the benefits of truly integrated (i.e. inter-programmatic and inter-sectoral) strategies. The findings of the present study may

provide useful information for such integrated strategies to overcome the public health burden of intestinal parasitic infections in Azaguié district in south Côte d’Ivoire in particular, and in other settings of the humid tropics.

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3. Accuracy of circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire

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3.1. Abstract

Background: Promising results have been reported for a circulating cathodic antigen (CCA) test for the diagnosis of *Schistosoma mansoni*. We assessed the accuracy of a commercially available CCA cassette test (designated CCA-A) and an experimental formulation (CCA-B) for *S. mansoni* diagnosis.

Methodology: We conducted a cross-sectional survey in three epidemiological settings of Côte d'Ivoire: settings A and B are endemic for *S. mansoni*, whereas *S. haematobium* co-exists in setting C. Overall, 446 school children, aged 8-12 years, submitted three stool and three urine samples. For *S. mansoni* diagnosis, stool samples were examined with triplicate Kato-Katz thick smears, whereas urine samples were tested with CCA-A. The first stool and urine samples were additionally subjected to an ether-concentration technique and CCA-B, respectively. Urine samples were examined for *S. haematobium* using a filtration method and for microhematuria using Hemastix dipsticks.

Principal Findings: Considering nine Kato-Katz thick smears as diagnostic 'gold' standard, the prevalence of *S. mansoni* in setting A, B and C was 32.9%, 53.1% and 91.8%, respectively. The sensitivity of triplicate Kato-Katz from the first stool and a single CCA-A test was 47.9% and 56.3% in setting A, 73.9% and 69.6% in setting B, and 94.2% and 89.6% in setting C. The respective sensitivity of a single CCA-B was 10.4%, 29.9% and 75.0%. The sensitivity of the ether-concentration technique for *S. mansoni* diagnosis was low (8.3-41.0%). The specificity of CCA-A was moderate (76.9-84.2%), whereas a high specificity was found for CCA-B (96.7-100%). The likelihood of a CCA-A color reaction increased with higher *S. mansoni* fecal egg counts (odds ratio: 1.07, $p < 0.001$). A concurrent *S. haematobium* infection or the presence of microhematuria did not influence the CCA-A test results for *S. mansoni* diagnosis.

Conclusion/Significance: CCA-A showed a sensitivity similar to triplicate Kato-Katz thick smears for *S. mansoni* diagnosis with no cross-reactivity to *S. haematobium* infections and microhematuria. The low sensitivity of CCA-B in our study area precludes its use for *S. mansoni* diagnosis.

3.2. Author summary

We aimed to assess the accuracy of a commercially available rapid diagnostic test for the detection of an infection with the blood fluke *Schistosoma mansoni*. In total, 446 school children from three different settings of south Côte d'Ivoire provided three stool and three urine samples. Stool samples were examined with the widely used Kato-Katz technique and analyzed with a microscope for *S. mansoni* eggs. Urine samples were examined with a filtration method for *S. haematobium* eggs and with a rapid diagnostic test for *S. mansoni* that is based on detecting circulating cathodic antigens (CCA). We used a commercially available test (designated CCA-A) and an experimental formulation (CCA-B). Examination of nine Kato-Katz thick smears per child revealed a prevalence of *S. mansoni* in the three settings of 32.9%, 53.1%, and 91.8%. The sensitivity of triplicate Kato-Katz from the first stool sample was comparable to a single CCA-A (47.9-94.2% vs. 56.3-89.6%), and significantly higher than the sensitivity of a single CCA-B test (10.4-75.0%). CCA-A showed a considerably lower specificity than CCA-B (76.9-84.2% vs. 96.7-100%). In the settings studied in south Côte d'Ivoire, the CCA-A test holds promise for the diagnosis of *S. mansoni*, whereas results with CCA-B were suboptimal.

3.3. Introduction

There is growing awareness, political commitment, and financial resources to control neglected tropical diseases (NTDs) (Hotez et al., 2007; Utzinger et al., 2009; Zhang et al., 2010). Preventive chemotherapy that is the repeated large-scale administration of drugs to at-risk populations, has become the key strategy for the control of several NTDs, including schistosomiasis (WHO, 2006; Utzinger et al., 2009; Hotez et al., 2008). Although the issue of diagnosis has received only token attention in the current era of preventive chemotherapy, its importance must be emphasized for rapid identification of high-risk communities warranting regular treatment, appraisal of drug efficacy, monitoring progress of control interventions, and improved patient management (Bergquist et al., 2009; Johansen et al., 2010; Utzinger et al., 2011). With regard to intestinal schistosomiasis due to *Schistosoma mansoni* and *S. japonicum*, the Kato-Katz technique is the most widely used diagnostic approach in epidemiological surveys (Katz et al., 1972; Utzinger et al., 2011). Although the Kato-Katz technique is relatively simple to perform, it requires a minimum of equipment (i.e., microscope, chemicals, and test kit material) and well-trained laboratory technicians (Speich et al., 2010). Moreover, a shortcoming of the Kato-Katz technique is the only low-to-

moderate sensitivity for *S. mansoni* diagnosis in low endemicity areas (de Vlas and Gryseels, 1992; Utzinger et al., 2001; Glinz et al., 2010). Hence, multiple Kato-Katz thick smears are required to enhance sensitivity (Utzinger et al., 2000), but this poses operational challenges and strains financial resources.

The detection of circulating antigen of *S. mansoni* in urine has been suggested as an alternative to the Kato-Katz technique (van Dam et al., 2004; Stothard, 2009; Shane et al., 2011). Indeed, both circulating anionic antigen (CAA) and circulating cathodic antigen (CCA) can be detected in sera and urine of individuals infected with *S. mansoni* (Utzinger et al., 2011). Both antigens are sensitive and specific to the antibodies and correlate with the presence and intensity of infection (Legesse and Erko, 2008). Antigen detection in urine using a rapid diagnostic test (RDT) based on an enzyme-linked immunosorbent assay (ELISA) technique is potentially useful and non-invasive and could change the management of infected individuals, particularly at the peripheral level in endemic countries where microscopes and qualified laboratory technicians are often not available (Bergquist et al., 2009; Johansen et al., 2010). A point-of-contact (POC) CCA urine test has been developed for the diagnosis of *S. mansoni* (Polman et al., 2000; van Lieshout et al., 2000), which is now commercially available as a RDT either in dipstick or cassette form. In view of promising results obtained thus far (Stothard et al., 2006; Shane et al., 2011), the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) initiated a multi-country study to assess the accuracy of a commercially available CCA cassette test for the diagnosis of *S. mansoni*.

The study reported here is part of this multi-country evaluation. We assessed the accuracy of a commercially available CCA cassette test (designated CCA-A) for *S. mansoni* diagnosis. Additionally, we employed an experimental formulation of the test (CCA-B). Nine Kato-Katz thick smears from each participant served as diagnostic ‘gold’ standard. In addition, our team employed the ether-concentration method on sodium acetate-acetic acid-formalin (SAF)-fixed stool samples for the diagnosis of *S. mansoni*, urine filtration for the identification of *S. haematobium* eggs and Hemastix dipsticks for the detection of microhematuria in urine. The study was carried out in south Côte d’Ivoire, in three settings where *S. mansoni* is endemic at different levels, whereas *S. haematobium* co-exists in one of the settings.

3.4. Methods

3.4.1. Ethics statement

The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland) and was cleared by the ethics committees of Basel (EKBB; reference no. 377/09) and Côte d'Ivoire (reference no. 1993 MSHP/CNER). District health and education authorities, village chiefs, parents/legal guardians, and participating children were informed about the purpose and procedures of the study. Parents/legal guardians provided written informed consent for their children to participate. Additionally, all children assented orally. Participation was voluntary and children could withdraw at any time without further obligation. All parasitological results were coded and treated confidentially. At the end of the study, children attending the schools involved in this study were treated with praziquantel (single 40 mg/kg oral dose) and albendazole (single 400 mg oral dose) free of charge, irrespective of the child's helminth infection status (WHO, 2002)

3.4.2. Study area and population

In October/November 2010, we carried out a cross-sectional survey in three epidemiological settings in the district of Azaguié, south Côte d'Ivoire. Azaguié is located approximately 40 km north of Abidjan, the economic capital of Côte d'Ivoire. The settings were selected after a pre-screening done in 10 schools. For the pre-screening, in each school, 25 children were randomly selected. All children attending grades 3-5 (CE1, CE2, and CM1) were given a unique number, lots including all numbers were closed and placed in a box, and finally 25 lots per school were drawn. The selected children provided a single stool and a single urine sample, which were examined for *S. mansoni* with triplicate Kato-Katz thick smears and *S. haematobium* with a single filtration, respectively. Based on this pre-screening, we selected the following sites, according to SCORE guidelines: setting A, low *S. mansoni* endemicity (i.e., prevalence: 10-24%); setting B, moderate *S. mansoni* endemicity (prevalence: 25-49%); and setting C, co-endemic for *S. mansoni* and *S. haematobium*.

3.4.3. Sample size

According to the literature, a single Kato-Katz thick smear for diagnosis of *S. mansoni* in low endemicity settings has a sensitivity of only 20-30% (Booth et al., 2003; Raso et al., 2007). However, since our study was to be carried out in both low and moderate endemicity settings,

we assumed that a single Kato-Katz thick smear has a maximum sensitivity of 60%. The sensitivity of the CCA test is reported to be 80% or higher (van Dam et al., 2004; Raso et al., 2007; Legesse and Erko, 2007). Using these sensitivity estimates, a significance level of 5%, and a power of 80%, our sample size of complying children was calculated at 90. Assuming a compliance of 70% for the submission of each of three requested stool samples, the number of children to be included in each study setting was at least 199. To achieve this sample size, we selected by computer-based randomization 220 children aged 8-12 years from readily available school lists of Abbé-Begnini (setting A), Azaguié Gare (setting B), and M'Bromé/Makouguié (setting C).

3.4.4. *Field procedures*

The purpose and procedures of the study were explained to the village authorities, the school directors, and the teachers of the selected schools. Teachers were invited to prepare class lists, including names, sex, and age of the children attending grades 3-5. Next, the study was explained to the children in lay terms and they were provided with an information and consent sheet with further details of the study and children and parents' rights. Children who submitted a written informed consent from their parents/guardians and assented orally themselves were given a 125 ml plastic container labeled with a unique identifier (ID). Children were invited to return the containers filled with a fresh lime-sized morning stool sample the following day. Upon collection of the filled container, a new empty container was handed out for stool collection on the next day. This procedure was repeated over a week until most children had submitted a total of three stool samples. Each day, between 10:00 and 12:00 hours, participating children were provided with another empty container labeled with the respective ID for collection of urine samples.

3.4.5. *Laboratory procedures*

Stool and urine samples were transferred to a laboratory at the Université de Cocody and processed the same day. From each stool sample, triplicate Kato-Katz thick smears were prepared, using 41.7 mg templates, following standard protocols (Katz et al., 1972). In brief, triplicate Kato-Katz thick smears were prepared on microscope slides, labeled with a child's ID plus letter A, B, or C. Slides were allowed to clear for at least 30 min before quantitative examination under a microscope by experienced laboratory technicians. The number of *S. mansoni* and other helminth eggs (e.g., *Ascaris lumbricoides*, hookworm, and *Trichuris*

trichiura) was counted and recorded for each species separately. For quality control, 10% of the Kato-Katz thick smears were re-examined by a senior technician.

In addition, from the second day stool sample, ~1 g of feces was weighed into plastic vials containing 10 ml of a SAF solution. Within 8 weeks, the SAF-fixed stool samples were processed with the ether-concentration method, following a standard protocol (Utzinger et al., 2010; Glinz et al., 2010). In brief, the stool-SAF solution was rigorously shaken and then poured through medical gauze placed on a plastic funnel into a conical glass tube. The conical tubes were centrifuged for 1 min at 500 x g. Subsequently, the supernatant was discarded and 7 ml of 0.85% sodium chloride (NaCl) solution and 2-3 ml ether were added to the pellet. Tubes were closed with a rubber stopper, manually shaken for ~30 sec and then centrifuged for 5 min at 500 x g. This procedure leads to the separation of the suspension in four layers. The three top layers were discarded and the complete sediment layer was placed on a microscope slide, covered with a slip and subsequently examined under a microscope for helminth eggs (i.e., *S. mansoni* and soil-transmitted helminths) and intestinal protozoon cysts.

All urine samples were subjected to CCA-A. The first urine sample was additionally subjected to CCA-B. Both CCA urine cassette assays were obtained from Rapid Medical Diagnostics (Pretoria, South Africa) and performed at ambient temperature, following the manufacturer's instructions. Briefly, one drop of urine was added to the well of the testing cassette and allowed to absorb. Once fully absorbed, one drop of buffer (provided with the CCA test kits) was added. The test results were read 20 min after adding the buffer. In case the control bands did not develop, the test was considered as invalid. Valid test were scored as either negative or positive, the latter further stratified into 1+, 2+, or 3+ according to the visibility of the color reaction. All tests were read independently by two blinded investigators and in case of discordant results discussed with a third independent investigator until agreement was reached.

In addition to the CCA cassettes, each urine sample was subjected to a filtration method for *S. haematobium* egg counts and to a Hemastix dipstick (Siemens Healthcare Diagnostics GmbH; Eschborn, Germany) for microhematuria assessment. In brief, samples were shaken, and 10 ml of urine filtered through a 13-mm diameter small meshed filter (20 µm; Sefar AG; Heiden, Switzerland), which was then placed on a labeled slide and examined under a microscope for *S. haematobium* eggs (Ayele et al., 2008). For appraisal of microhematuria, a Hemastix dipstick was soaked in urine, left in the open air for 1 min, before scoring according to the manufacturer's instructions.

3.4.6. Statistical analysis

Data were entered twice in a Microsoft Excel spreadsheet, transferred in EpiInfo version 6.4 (Centers for Disease Control and Prevention; Atlanta, GA, USA) and validated. Statistical analyses were done with STATA version 10 (Stata Corp.; College Station, TX, USA).

Only those children who had complete data records were included in the final analysis (i.e., nine Kato-Katz thick smears, a single ether-concentration, three CCA-A, one CCA-B, three urine filtrations, and three Hemastix dipsticks). To obtain a standardized measure of infection intensity, expressed as eggs per gram of stool (EPG), for each individual, we calculated the arithmetic mean *S. mansoni* fecal egg counts (FECs) from the nine Kato-Katz thick smears and multiplied by a factor 24. Infection intensity of *S. mansoni* was classified into light (1-99 EPG), moderate (100-399 EPG), and heavy (≥ 400 EPG). Egg counts of *S. haematobium* were utilized to stratify into light (1-49 eggs/10 ml of urine) and heavy infection intensities (≥ 50 eggs/10 ml of urine) (WHO, 2006).

The strength of agreement between nine Kato-Katz thick smears and triplicate CCA-A, one CCA-B, and one ether-concentration for each endemicity setting was assessed by kappa statistics (κ), as follows: $\kappa < 0$ indicating no agreement, $\kappa = 0-0.2$ indicating poor agreement, $\kappa = 0.21-0.4$ indicating fair agreement, $\kappa = 0.41-0.6$ indicating moderate agreement, $\kappa = 0.61-0.8$ indicating substantial agreement, and $\kappa = 0.81-1.0$ indicating almost perfect agreement (Landis and Koch, 1977; Cohen, 1960).

As proposed by the SCORE secretariat, the results from nine Kato-Katz thick smears were considered our ‘gold’ standard. We determined the sensitivity (proportion of true-positives detected by the test) and specificity (proportion of true-negatives detected by the test) of single and multiple tests. As with some of our previous work, we used a second ‘gold’ standard by considering a positive test result (regardless of the test) as true-positive (Landis and Koch, 1977; Steinmann et al., 2008; Becker et al., 2011). Hence, we combined results from all tests (i.e., nine Kato-Katz thick smears plus triplicate CCA-A, one CCA-B, and one ether-concentration) and therefore maximized specificity.

We employed an ordinal logistic regression approach, which is an extension of the general linear model to ordinal categorical outcomes to assess the correlation between CCA-A and CCA-B color reaction categories and *S. mansoni* FECs. The arithmetic mean FEC of three Kato-Katz thick smears per stool sample per day served as continuous explanatory variable, whereas the color reaction of the CCA test was considered as categorical outcome. This statistical procedure was also used to compare between the CCA test results considered

as categorical outcome, and different infection intensity categories of *S. mansoni* (i.e., light, moderate, and heavy) utilized as categorical explanatory variables.

A logistic regression was performed to assess the association between CCA-A and CCA-B test results, expressed as binary outcome variable (negative/positive) with *S. haematobium* egg count as continuous explanatory variable and microhematuria as categorical explanatory variable among children without a *S. mansoni* infection.

3.5. Results

3.5.1. Study adherence

Figure 3.1 shows the adherence of school children to provide multiple stool and urine samples for a suite of diagnostic tests for detection of *S. mansoni* and *S. haematobium* infection. Overall, 674 school children aged 8-12 years were enrolled with slightly more boys than girls (343 vs. 331). The number of children in settings A, B and C was 234, 220 and 220, respectively. At least one stool and one urine sample was provided by 223, 178 and 206 children in settings A, B and C, respectively. Overall, 465 children submitted three stool samples, which were subjected to triplicate Kato-Katz thick smears. Results from a single ether-concentration method were available for 555 children. Three CCA-A test results were available for 489 children, whereas 545 children had the first urine sample additionally subjected to a CCA-B test. Finally, three urine filtrations for *S. haematobium* diagnosis and three Hemastix dipstick tests for appraisal of microhematuria were done for 489 children.

Results on three stool samples (examined with nine Kato-Katz thick smears and a single ether-concentration) and three urine samples (examined with three CCA-A, one CCA-B, three urine filtrations and three Hemastix dipsticks) were available from a total of 446 children. All further analysis focused on this cohort of children.

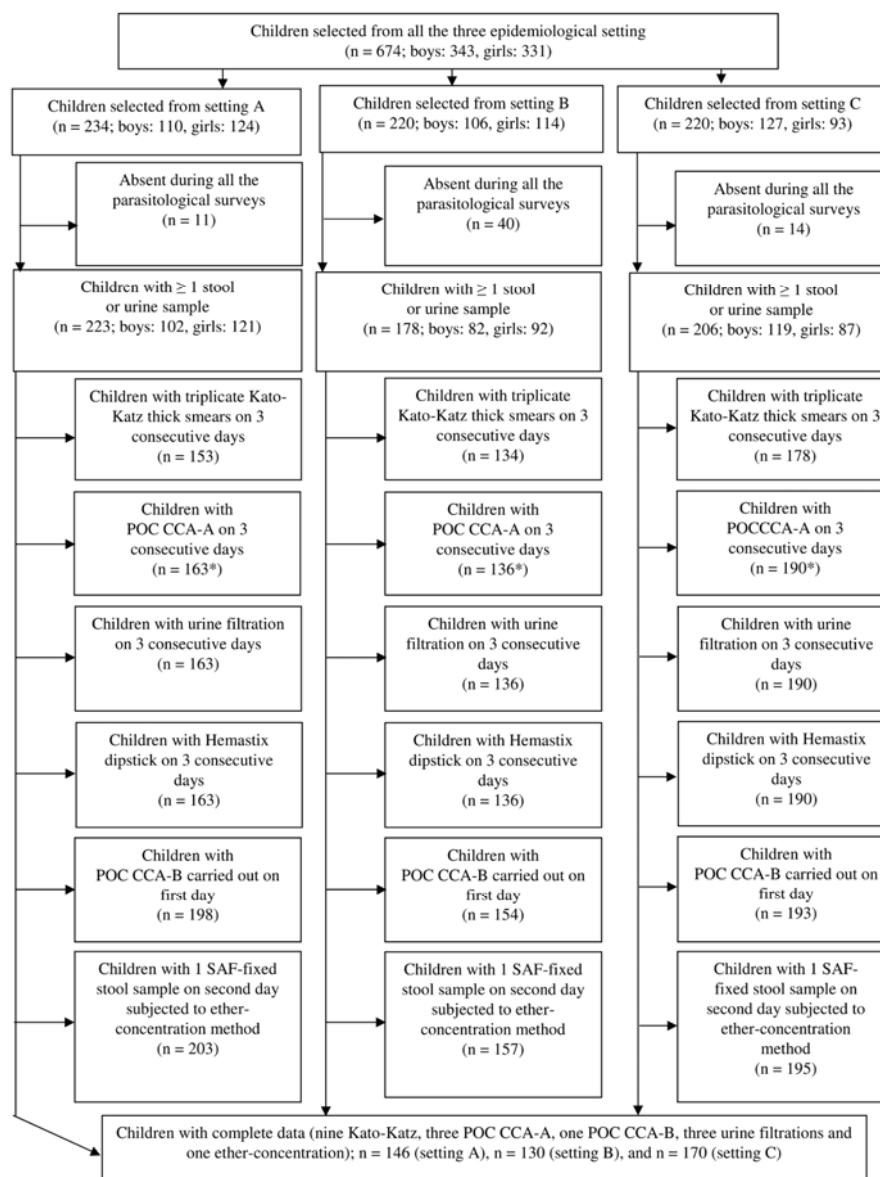


Figure 3.1: Flowchart showing study participation, stratified by epidemiological setting. Flowchart detailing the study participation and adherence of children for multiple stool and urine submissions for diagnosis of *S. mansoni* and *S. haematobium* infection in Azaguié, south Côte d'Ivoire, in October and November 2010. According to nine Kato-Katz thick smear examinations, the prevalence of *S. mansoni* in setting A, B and C was 32.9%, 53.1% and 91.8%, respectively. In setting C, *S. haematobium* is co-endemic.

3.5.2. *S. mansoni* and *S. haematobium* Infection

Table 3.1 shows the number of children examined and those positive for *S. mansoni* and *S. haematobium*, as assessed by different diagnostic approaches, stratified by study setting.

3.5.2.1. Kato-Katz Technique and Ether-Concentration Method

In setting A, B and C, according to nine Kato-Katz thick smears examined per child, the observed prevalence of *S. mansoni* was 32.9%, 53.1%, and 91.8%, respectively. In settings A and B, most of the infections were of light intensity (96.2% and 68.1%, respectively), whereas in setting C, three-quarter of the children had moderate or heavy infection intensities (76.2%). The lowest arithmetic mean FEC was found in setting A (17.4 EPG, 95% CI: 0-38.9 EPG) and the highest arithmetic mean FEC in setting C (482.8 EPG, 95% CI: 388.1-577.4 EPG). In setting B, the arithmetic mean FEC was 62.4 EPG (95% CI: 36.2-88.5 EPG).

Considerably lower *S. mansoni* prevalence estimates were obtained after subjecting a single stool sample to an ether-concentration method, 4.1%, 6.9% and 38.2% in setting A, B and C, respectively.

3.5.2.2. CCA test results

In setting A, B and C, the respective prevalence of *S. mansoni* based on triplicate CCA-A tests were 33.8%, 48.8% and 85.9%. Out of the 570 CCA-A tests performed on three consecutive days in setting A, a total of 141 showed a positive color reaction, most of which were classified as 1+ (n = 105, 74.5%). Reactions of 2+ and 3+ were seen in 24 (17.0%) and 13 (9.2%) of the tests, respectively. In setting B, 460 CCA-A tests were performed. Overall, 170 tests showed a positive reaction: 99 were judged 1+ (58.2%), 44 considered 2+ (25.9%) and the remaining 26 as 3+ (15.3%). Finally, in setting C, 76 (15.8%), 150 (31.1%) and 256 (53.1%) of the CCA-A tests performed were classified as 1+, 2+ and 3+, respectively.

The prevalence of *S. mansoni* according to a single CCA-B in setting A, B and C was 3.4%, 17.2% and 69.8%, respectively. In setting A, the five positive CCA-B tests were all judged 1+. In setting B, one CCA-B test result was considered 2+, whereas the remaining 21 test results were classified as 1+. Finally, in setting C, there were 116 CCA-B test results considered as 1+ (87.2%) and 17 as 2+ (12.8%).

3.5.2.3. Urine filtration and hemastix results

In settings A, B and C, according to triplicate urine filtrations, the prevalence of *S. haematobium* was 4.1%, 0.8% and 65.3% in setting A, B and C, respectively. With regard to Hemastix dipstick results, after exclusion of trace results, the prevalence of microhematuria in settings A, B and C was 5.3%, 6.7% and 44.6%. Traces of microhematuria were additionally found in 33, 34 and 78 urines tested in setting A, B and C, respectively.

Table 3.1: Prevalence of *S. mansoni* and *S. haematobium* according to different diagnostic approaches, stratified by epidemiological setting.

Diagnostic approach	Setting A			Setting B			Setting C		
	No. of children tested	No. of children positive	% positive (CI*)	No. of children tested	No. of children positive	% positive (CI*)	No. of children tested	No. of children positive	% positive (CI*)
	<i>S. mansoni</i> diagnosis								
Nine Kato-Katz thick smears	146	48	32.9 (25.2-40.6)	130	69	53.1 (44.4-61.8)	170	156	91.8 (87.6-95.9)
Three CCA-A**	146	49	33.8 (26.0-41.6)	130	60	48.8 (39.8-57.7)	170	141	85.9 (80.6-91.3)
One CCA-B*	146	5	3.4 (0.3-4.7)	130	22	17.2 (10.6-23.8)	170	117	68.8 (61.8-75.9)
One ether concentration	146	6	4.1 (0.8-7.4)	130	9	6.9 (2.5-11.3)	170	65	38.2 (30.8-45.6)
<i>S. haematobium</i> diagnosis									
Three Hemastix dipsticks (excluding trace results)	113	6	5.3 (1.0-9.5)	86	5	6.7 (1.4-12.1)	92	41	44.6 (32.2-54.9)
Three urine filtrations	146	6	4.1 (0.8-7.4)	130	1	0.8 (0.0-2.2)	170	111	65.3 (58.1-72.1)

The study was carried out in three epidemiological settings of south Côte d'Ivoire in October and November 2010. Triplicate Kato-Katz thick smears from the first collected stool sample, nine Kato-Katz thick smears from three stool samples, one CCA-A from the first collected urine sample, three CCA-A tests from three urine samples, one CCA-B test from the first collected urine sample, and one ether-concentration test on SAF-fixed stool samples from the second collected stool sample were used for the diagnosis of *S. mansoni*. Three urine filtrations were employed for *S. haematobium* diagnosis and three Hemastix dipsticks were used for microhematuria appraisal.

* Exact 95% confidence interval

** In settings A, B and C, there were 2, 6 and 7 tests considered invalid, and hence not taken into account for prevalence calculations

3.5.3. *Association between Kato-Katz and CCA test results*

As indicated in Table 3.2, our ordinal logistic regression analysis showed that for an increase of *S. mansoni* infection intensity by 1 EPG, the likelihood of a stronger color reaction of the CCA-A (odds ratio (OR) = 1.07) and the CCA-B (OR = 1.03) is significant (both $p < 0.001$). When *S. mansoni* FECs were not considered as continuous, but stratified according to pre-set thresholds into no, light, moderate and heavy infection intensity, we found that for each increase in infection intensity category, the likelihood of a stronger color reaction of both CCA-A (OR = 36.5) and CCA-B (OR = 25.2) is highly significant (both $p < 0.001$). Figure 3.2 shows the correlation between infection intensity classes according to pre-set thresholds (WHO, 2002) and the percentage of infected individuals as determined by a single or triplicate CCA-A and a single CCA-B.

Table 3.2: Correlation of CCA test with schistosome infection and microhematuria (n = 526).

Test	Association	Adjusted OR (95% CI)	P-value
CCA-A			
(color categories)	<i>S. mansoni</i> egg count	1.07 (1.05, 1.09)	<0.001
	<i>S. mansoni</i> infection categories	36.50 (27.35, 48.77)	<0.001
CCA-A (pos/neg)	<i>S. haematobium</i> egg count	1.09 (0.97, 1.21)	0.121
	Hematuria trace	0.39 (0.09, 1.64)	0.200
	Hematuria moderate (2+)	1.09 (0.17, 7.00)	0.923
	Hematuria heavy (3+)	0.90 (0.12, 6.99)	0.195
CCA-B			
(color categories)	<i>S. mansoni</i> egg count	1.03 (1.01, 1.04)	<0.001
	<i>S. mansoni</i> infection categories	25.20 (15.83, 39.95)	<0.001
CCA-B (pos/neg)	<i>S. haematobium</i> egg count	NA*	
	Hematuria trace	NA*	
	Hematuria moderate (2+)	NA*	
	Hematuria heavy (3+)	NA*	

* NA: not applicable due to the small number of children without *S. mansoni* infection

Ordinal logistic regression was used to assess the correlation between CCA test categories (0, 1+, 2+, 3+) as outcome and *S. mansoni* egg count or *S. mansoni* infection categories (low, moderate, and heavy) as explanatory variable. Category “low” was used as baseline for comparison of other categories.

Logistic regression was applied to assess the correlation between CCA test results expressed as binary variables (positive/negative) and *S. haematobium* egg counts and microhematuria categories (trace, 1+, 2+, 3+). Category “1+” was used as baseline for comparison of other categories.

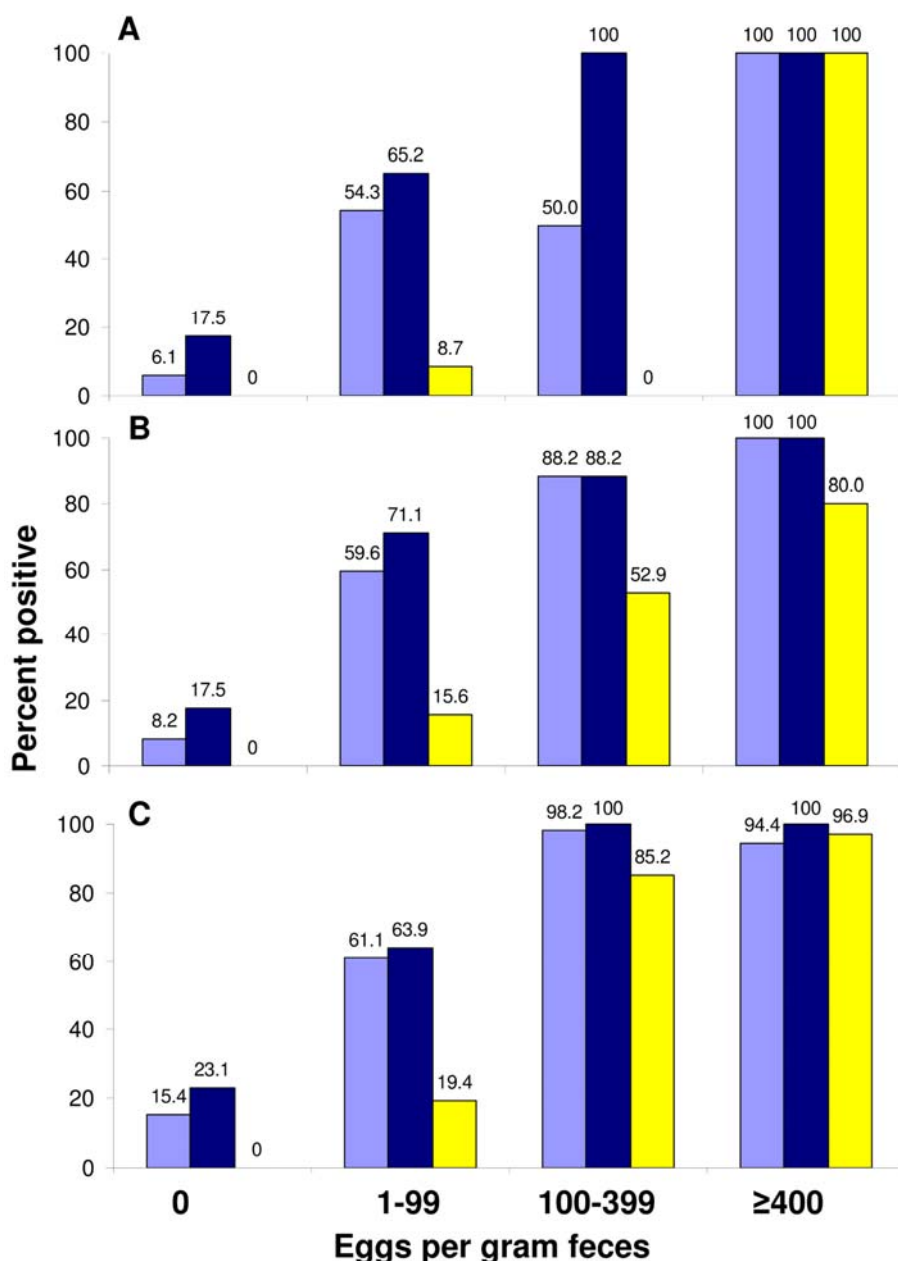


Figure 3.2: Correlation between Kato-Katz and CCA for *S. mansoni* diagnosis. Figure showing the correlation between the prevalence and intensity (stratified by intensity class) of *S. mansoni* infections, as determined by a single or triplicate CCA-A (light blue and dark blue bar, respectively), and a single CCA-B (yellow bar), stratified by study setting. According to nine Kato-Katz thick smear examinations, the prevalence of *S. mansoni* in setting A, B and C was 32.9%, 53.1% and 91.8%, respectively. In setting C, *S. haematobium* is co-endemic.

3.5.4. *Diagnosis accuracy of different tests*

3.5.4.1. Agreement of the diagnostic assays

Table 3.3 shows the agreement between different diagnostic approaches and our first ‘gold’ standard (i.e., nine Kato-Katz thick smears derived from three stool samples) for the diagnosis of *S. mansoni*, stratified by study setting. The agreement between nine Kato-Katz thick smears and (i) a single Kato-Katz from the first stool sample was fair in setting A ($\kappa = 0.31$) and moderate in settings B and C ($\kappa = 0.44-0.45$), (ii) triplicate Kato-Katz from a single stool sample was moderate in settings A ($\kappa = 0.55$), and substantial in settings B and C ($\kappa = 0.73$), (iii) a single CCA-A was moderate in settings A, B and C ($\kappa = 0.49-0.60$), (iv) three CCA-A tests was moderate in settings A and C ($\kappa = 0.49-0.51$) and substantial in setting B ($\kappa = 0.61$), (v) a single CCA-B was poor in setting A ($\kappa = 0.14$) and fair in settings B and C ($\kappa = 0.26-0.33$), and (vi) a single ether-concentration method was poor for all three settings ($\kappa = 0.08-0.12$).

Table 3.3: Agreement between different techniques for the diagnosis of *S. mansoni*.

Diagnostic approach	Test result	Test											
		Setting A				Setting B				Setting C			
		Nine Kato-Katz thick smears				Nine Kato-Katz thick smears				Nine Kato-Katz thick smears			
		Positive	Negative	K*	P-values	Positive	Negative	K*	P-values	Positive	Negative	K*	P-values
One Kato-Katz thick smear	Positive	12	0			32	0			129	0		
	Negative	36	98	0.31	<0.001	37	61	0.45	<0.001	27	14	0.44	<0.001
Three Kato-Katz thick smears	Positive	23	0			51	0			147	0		
	Negative	25	98	0.55	<0.001	18	61	0.73	<0.001	9	14	0.73	<0.001
One CCA-A	Positive	27	6			48	5			138	2		
	Negative	21	92	0.54	<0.001	21	56	0.60	<0.001	16	11	0.49	<0.001
Three CCA-A	Positive	32	17			51	9			138	3		
	Negative	16	80	0.49	<0.001	15	48	0.61	<0.001	13	10	0.51	<0.001
One CCA-B	Positive	5	0			20	2			117	0		
	Negative	43	98	0.14	0.001	47	59	0.26	<0.001	39	14	0.33	<0.001
One ether-concentration	Positive	4	2			9	0			64	1		
	Negative	44	96	0.08	0.044	60	61	0.12	0.002	92	13	0.09	0.008

The study was carried out in three settings in south Côte d'Ivoire in October and November 2010. The κ -agreement of a single Kato-Katz thick smear from the first collected stool sample, triplicate Kato-Katz thick smears from the first collected stool sample, one CCA-A from the first collected urine sample, three CCA-A tests from three collected urine samples, one CCA-B test from the first collected urine sample, and one ether-concentration test on SAF-fixed stool samples from the second collected stool sample versus our diagnostic 'gold' standard of nine Kato-Katz thick smears (triplicate Kato-Katz thick smears from each of three stool samples) for the diagnosis of *S. mansoni* was calculated.

* stands for kappa, $\kappa < 0$ indicating no agreement, $\kappa = 0-0.2$ indicating poor agreement, $\kappa = 0.21-0.4$ indicating fair agreement, $\kappa = 0.41-0.6$ indicating moderate agreement, $\kappa = 0.61-0.8$ indicating substantial agreement, and $\kappa = 0.81-1.0$ indicating almost perfect agreement (Landis and Koch, 1977; Cohen, 1960).

3.5.5. Sensitivity and specificity of the diagnostic techniques

The sensitivity and specificity of the different diagnostic tests were determined for each of our two ‘gold’ standards (Table 3.4). In setting A, if the combined results of nine Kato-Katz thick smears were used as ‘gold’ standard, very low sensitivities of only 8.3% for a single ether-concentration test and 10.4% for a single CCA-B were determined. The highest sensitivity was revealed for triplicate (66.7%) CCA-A tests followed by duplicate (60.4%) and a single CCA-A test (56.3%).

In setting B, the lowest sensitivity of 13.0% was determined for a single ether-concentration test. The highest sensitivity was found for duplicate or triplicate CCA-A (both = 77%). Single CCA-A (69.6%), showed a slightly lower sensitivity than triplicate Kato-Katz thick smears (73.9%) from a single stool sample.

In setting C, the ether-concentration showed the lowest sensitivity (41.0%) for diagnosis of *S. mansoni*. A single (89.6%) and duplicate or triplicate CCA-A tests (both = 91%) did not show a higher sensitivity than triplicate Kato-Katz thick smears (94.2%).

Considering nine Kato-Katz thick smears as ‘gold’ standard, the specificity of triplicate CCA-A test was 82.5% (setting A), 84.2% (setting B), and 76.9% (setting C). The respective specificity of the CCA-B test was 100%, 96.7% and 100%.

Even when using a more rigorous diagnostic ‘gold’ standard (i.e., the combined results of nine Kato-Katz thick smears, plus triplicate CCA-A, a single CCA-B, and a single ether-concentration), very similar sensitivities were obtained.

Table 3.4: Sensitivity and specificity of different tests for the diagnosis of *S. mansoni*.

	Setting A		Setting B		Setting C	
	Sensitivity, % (CI*)	Specificity, % (CI*)	Sensitivity, % (CI*)	Specificity, % (CI*)	Sensitivity, % (CI*)	Specificity, % (CI*)
Nine Kato-Katz thick smears as ‘gold’ standard						
Single Kato-Katz	25.0 (13.6, 39.6)	100 (96.3, 100)	46.4 (34.3, 58.8)	100 (94.1, 100)	82.7 (75.8, 88.3)	100 (76.8, 100)
Duplicate Kato-Katz	29.2 (17.0, 44.1)	100 (96.3, 100)	55.1 (42.6, 67.1)	100 (94.1, 100)	86.5 (80.2, 91.5)	100 (76.8, 100)
Triplicate Kato-Katz	47.9 (33.3, 62.8)	100 (96.3, 100)	73.9 (61.9, 83.7)	100 (94.1, 100)	94.2 (89.3, 97.3)	100 (76.8, 100)
Single CCA-A	56.3 (41.2, 70.5)	93.9 (87.1, 97.7)	69.6 (57.3, 80.1)	91.8 (81.9, 97.3)	89.6 (83.5, 93.7)	84.6 (54.6, 98.6)
Double CCA-A	60.4 (45.3, 74.2)	88.7 (80.6, 94.2)	77.3 (65.3, 86.7)	84.2 (72.1, 92.5)	91.4 (85.7, 95.3)	76.9 (46.2, 95.0)
Triplicate CCA-A	66.7 (51.6, 79.6)	82.5 (73.4, 89.4)	77.3 (65.3, 86.7)	84.2 (72.1, 92.5)	91.4 (85.7, 95.3)	76.9 (46.2, 95.0)
One CCA-B	10.4 (3.4, 22.7)	100 (96.3, 100)	29.9 (19.3, 42.3)	96.7 (88.7, 100)	75.0 (67.4, 81.6)	100 (76.8, 100)
One ether-concentration	8.3 (2.3, 20.0)	98.0 (92.9, 99.8)	13.0 (6.1, 23.3)	100 (94.1, 100)	41.0 (33.2, 49.2)	92.9 (66.1, 99.8)
Combined results as ‘gold’ standard						
Single Kato-Katz	17.9 (9.6, 29.2)	100 (95.4, 100)	40.0 (28.9, 52.0)	100 (92.3, 100)	80.5 (73.4, 86.5)	100 (69.2, 100)
Duplicate Kato-Katz	20.9 (11.9, 32.6)	100 (95.4, 100)	48.0 (36.3, 59.8)	100 (92.3, 100)	84.4 (77.7, 89.8)	100 (69.2, 100)
Triplicate Kato-Katz	34.3 (23.2, 46.9)	100 (95.4, 100)	64.0 (52.1, 74.8)	100 (92.3, 100)	92.2 (86.8, 95.9)	100 (69.2, 100)
Single CCA-A	47.8 (35.4, 60.3)	100 (95.4, 100)	65.3 (53.5, 76.0)	100 (92.3, 100)	89.0 (82.9, 93.4)	100 (69.2, 100)
Duplicate CCA-A	73.1 (60.9, 83.2)	100 (95.4, 100)	77.3 (66.2, 86.2)	100 (92.3, 100)	91.6 (86.0, 95.4)	100 (69.2, 100)
Triplicate CCA-A	73.1 (60.9, 83.2)	100 (95.4, 100)	77.3 (66.2, 86.2)	100 (92.3, 100)	91.6 (86.0, 95.4)	100 (69.2, 100)
One CCA-B	7.5 (2.5, 16.6)	100 (95.4, 100)	28.0 (18.2, 39.6)	100 (92.3, 100)	72.7 (65.0, 79.6)	100 (69.2, 100)
One ether-concentration	8.9 (3.4, 18.5)	100 (95.4, 100)	10.7 (4.7, 19.9)	100 (92.3, 100)	39.6 (31.8, 47.8)	100 (69.2, 100)

The study was carried out in three epidemiological settings of south Côte d’Ivoire in October and November 2010. Two different diagnostic ‘gold’ standards were applied to calculate sensitivity and specificity, namely (i) the combined results of nine Kato-Katz thick smears, and (ii) the combined results of all tests able to diagnose *S. mansoni* infections (i.e., nine Kato-Katz thick smears, one ether-concentration, three CCA-A, and one CCA-B)

*Exact 95% confidence interval

3.5.6. Effect of concurrent *S. haematobium* infection

Table 3.2 shows that, if only *S. mansoni*-negative children were included in a logistic regression analysis and adjustments were made for *S. haematobium* egg counts and infection intensity classes, no significant association between the CCA-A positivity rate and *S. haematobium* egg counts was found (OR = 1.09; $p = 0.121$). There was also no significant association between the CCA-A positivity rate and microhematuria classes detected ($p > 0.05$). Due to the small number of children found positive with the CCA-B test no logistic regression analysis was performed.

3.6. Discussion

For the rapid identification of populations at highest risk of schistosomiasis that warrant preventive chemotherapy, as well as for monitoring progress of control interventions and new efforts toward elimination, assessment of drug efficacy, and improved patient management, the importance of an accurate diagnosis at the individual and population level must be emphasized (Bergquist et al., 2009; Becker et al., 2011; Johansen et al., 2010). The widely used Kato-Katz technique for the diagnosis of *S. mansoni* (and *S. japonicum*) has several shortcomings: in low endemicity settings this technique considerably underestimates the ‘true’ prevalence of infection (Yu et al., 2007; Lin et al., 2008; Utzinger et al.). Moreover, a minimum of equipment and well trained laboratory technicians are needed for quality results.

Promising results have been reported with a CCA urine dipstick and cassette test for the diagnosis of *S. mansoni* in different settings (Shane et al., 2011; Stothard et al., 2011). However, a shortcoming of the previous studies was the lack of a rigorous diagnostic ‘gold’ standard, as CCA test results were usually compared with single or duplicate Kato-Katz thick smears from one or two stool samples (Stothard et al., 2006; Ashton et al., 2011). Within the frame of a SCORE-funded multi-country study, we have now assessed the accuracy of a commercially available CCA urine cassette assay (CCA-A) and an experimental formulation (CCA-B) provided by the same manufacturer and tuned to have a higher specificity to run in parallel with the commercially available test in three epidemiological settings of south Côte d’Ivoire. Results of the CCA tests were compared with nine Kato-Katz thick smears (three stool samples, each subjected to triplicate Kato-Katz thick smears). Additionally, we performed a single ether-concentration test using SAF-fixed stool samples. The influence of *S. haematobium* infection and presence of microhematuria on the performance of the CCA test was determined. In all three settings, a single CCA-A showed a similarly high sensitivity

than triplicate Kato-Katz thick smears from a single stool sample, but both approaches missed a considerable number of infections when considering nine Kato-Katz thick smears as ‘gold’ standard. The sensitivity of a single CCA-B was significantly lower than that of triplicate Kato-Katz thick smears, particularly in settings A and B where the endemicity of *S. mansoni* was lower than in setting C. However, CCA-B showed a higher specificity than CCA-A.

The CCA-A seems to be an appropriate test for the diagnosis of *S. mansoni* in our study area in south Côte d’Ivoire where the prevalence of *S. mansoni* is above 25% and no recent control efforts have been implemented. Importantly, we did not detect any cross-reactivity of the CCA-A between *S. mansoni* and *S. haematobium*. This adds to the results of no cross-reactivity of the CCA cassette between *S. mansoni* and soil-transmitted helminths determined by Shane and colleagues in a recent study from Kenya (Shane et al., 2011). Furthermore, our study did not reveal a significant association between CCA-A positive results and microhematuria, as determined by Hemastix dipsticks, which relaxes the manufacturer’s indication that false-positive results can occur if an individual presents microhematuria. However, further studies in different settings are warranted to confirm that microhematuria or urinary tract infections are not negatively impacting on CCA test results. Also the ability of the CCA test to detect antigen of juvenile *Schistosoma* worms, which are not yet producing eggs, needs further investigation. Noteworthy, the sensitivity of 56.3% of a single CCA-A in the setting A with a *S. mansoni* prevalence of 32.9% (based on nine Kato-Katz thick smears) is considerably lower than the sensitivity of 96.3% detected with a single CCA cassette of the same manufacturer in a Kenyan setting with a similar prevalence (38.8%) (Shane et al., 2011). This difference might be explained by our more rigorous diagnostic approach, i.e., triplicate instead of duplicate Kato-Katz thick smears of three consecutive stool samples as ‘gold’ standard and by working in a slightly lower endemicity area. The sensitivity of a single CCA-A for *S. mansoni* diagnosis increased from 56.3% (setting A) to 69.6% (setting B) and 89.6% (setting C) in parallel to increasing prevalence (32.9% to 53.1% and finally to 91.8%), and corresponding mean FECs (17.4 EPG to 62.4 EPG and finally to 482.8 EPG). These findings emphasize the impact of higher prevalences and infection intensities on the positivity rate of the CCA. The strong association between the intensity of the color reaction of the CCA-A band and *S. mansoni* infection intensities according to FECs by the Kato-Katz method in our studies is in line with previous reports of the CCA dipstick and cassette (Legesse and Erko, 2008; Standley et al., 2010). The results of the experimental CCA-B formulation, which has been tested on a single urine sample from all children, are sub-optimal. Indeed, only low

sensitivities and a poor agreement with results of the Kato-Katz method were found, particularly in the lower endemicity areas (settings A and B). In our hands, despite high specificity, the CCA-B in its current formulation cannot be recommended for *S. mansoni* diagnosis in south Côte d'Ivoire.

The following issues speak for or against the application of the CCA-A versus the Kato-Katz method in helminth control programs or public health centers: at first view, in moderate-to-high-risk communities for *S. mansoni* infections as found in our study in Côte d'Ivoire (i.e., prevalence above 25%), the collection of a single stool sample and its examination with triplicate Kato-Katz thick smears seems to be an acceptable approach for *S. mansoni* diagnosis. The advantage of the Kato-Katz method is that it can concurrently detect other helminth species, such as the three main soil-transmitted helminths (i.e., *A. lumbricoides*, hookworm, and *T. trichiura*), which is not possible with the CCA. However, the Kato-Katz method requires a minimum of equipment, including a microscope, and well trained laboratory technicians who can identify helminth species-specific eggs in the thick smears. For application of the CCA-A, no additional equipment and only a minimum of training are needed. However, it only detects *S. mansoni* and no concurrent soil-transmitted helminth infections. The cost of a single cassette (approximately US\$ 2) is currently still out of reach of people at highest risk of intestinal schistosomiasis (i.e., poor rural dwellers in sub-Saharan Africa) (Shane et al., 2011). However, the cost of triplicate Kato-Katz thick smears is likely higher than a single CCA test (Speich et al., 2010). From a convenience and logistical point of view, the collection of urine samples for the CCA is more straightforward than collection of stool for the Kato-Katz method. Indeed, urine production is more convenient for the patient and can be done without special efforts on the spot and at the same day resulting in high compliance rates, while stool production is inconvenient and collection can render a second consultation necessary and thus further exacerbate costs (Legesse and Erko, 2007).

The performance and sensitivity of the CCA test in low-risk communities (prevalence below 10%), identified by the application of multiple Kato-Katz thick smears on stool samples collected over multiple days, remains to be elucidated. Noteworthy, our study intended to test the CCA in a setting with a *S. mansoni* prevalence of 10-24% as requested by SCORE. However, we observed a considerable increase in the prevalence of *S. mansoni* when not only applying triplicate Kato-Katz from a single stool sample as in the pre-screening, but nine Kato-Katz thick smears overall from three stool samples: the observed prevalence increased from 17% to 34% in setting A, and from 36% to 54% in setting B. Due to this

rigorous diagnostic approach we ended up with higher prevalences than initially anticipated. If the CCA test proves to be more sensitive than multiple Kato-Katz thick smears in settings characterized by low prevalence and intensity of *S. mansoni* infection intensities, it will be a most useful test. For example, in areas where intense helminth control efforts have diminished the prevalence and intensity of *S. mansoni* infections and control programs are focusing elimination, population screenings are necessary to identify remaining *S. mansoni* hot-spots for targeted anthelmintic treatment and other interventions. For these large-scale screenings the CCA-A would be an excellent tool due to its fast and easy application.

3.7. Conclusion

We conclude that in the current study area of south Côte d'Ivoire, where the prevalence and intensity of *S. mansoni* are still high, partially explained by the prior lack of control efforts, the CCA-A can become a useful method for *S. mansoni* diagnosis in health centers at the periphery and schistosomiasis control programs. On the other hand, while the specificity of the CCA-B test was high, its current formulation cannot be recommended for *S. mansoni* diagnosis. Clearly, there is a need to evaluate the CCA test in settings characterized by low *S. mansoni* prevalences and infection intensities to assess its potential role in schistosomiasis control programs progressing toward transmission control and local elimination and for reliable individual diagnosis.

3.8. Acknowledgments

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4. Accuracy of urine circulating cathodic antigen assay for *Schistosoma mansoni* diagnosis in preschool-aged children before and after treatment

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4.1. Abstract

Background: The Kato-Katz technique is widely used for the diagnosis of *Schistosoma mansoni*, but shows low sensitivity in light-intensity infections. We assessed the accuracy of a commercially available point-of-care circulating cathodic antigen (POC-CCA) cassette test for the diagnosis of *S. mansoni* in preschool-aged children before and after praziquantel administration.

Methodology: A 3-week longitudinal survey with a treatment intervention was conducted in Azaguié, south Côte d'Ivoire. Overall, 242 preschoolers (age range: 2 months to 5.5 years) submitted two stool and two urine samples before praziquantel administration, and 86 individuals were followed-up posttreatment. Stool samples were examined with duplicate Kato-Katz thick smears for *S. mansoni*. Urine samples were subjected to POC-CCA cassette test for *S. mansoni*, and a filtration method for *S. haematobium* diagnosis.

Principal Findings: Before treatment, the prevalence of *S. mansoni*, as determined by quadruplicate Kato-Katz, single CCA considering 'trace' as negative (t-), and single CCA with 'trace' as positive (t+), was 23.1%, 34.3% and 64.5%, respectively. Using the combined results (i.e., four Kato-Katz and duplicate CCA(t-)) as diagnostic 'gold' standard, the sensitivity of a single Kato-Katz, a single CCA(t-) or CCA(t+) was 28.3%, 69.7% and 89.1%, respectively. Three weeks posttreatment, the sensitivity of a single Kato-Katz, single CCA(t-) and CCA(t+) was 4.0%, 80.0% and 84.0%, respectively. The intensity of the POC-CCA test band reaction was correlated with *S. mansoni* egg burden (odds ratio = 1.2, $p = 0.04$).

Conclusions/Significance: A single POC-CCA cassette test appears to be more sensitive than multiple Kato-Katz thick smears for the diagnosis of *S. mansoni* in preschool-aged children before and after praziquantel administration. The POC-CCA cassette test can be recommended for the rapid identification of *S. mansoni* infections before treatment. Additional studies are warranted to determine the usefulness of POC-CCA for assessing drug efficacy and monitoring the impact of control interventions.

4.2. Author summary

The strategy to control morbidity due to infection with the blood fluke *Schistosoma mansoni* is to regularly treat school children with the drug praziquantel. Recent studies suggest that in highly endemic areas preschoolers might need to be included in such deworming activities. An accurate diagnosis is important to assess the extent of preschool-aged children needing

treatment, but the widely used Kato-Katz technique does not detect all infections. We assessed the accuracy of a point-of-care (POC) test that is based on the detection of the fluke's circulating cathodic antigen (CCA) in children's urine. We obtained two stool and two urine samples from 242 preschoolers in Côte d'Ivoire before and 86 children after praziquantel treatment. Stool samples were examined with the Kato-Katz technique and urine samples with the POC-CCA test for *S. mansoni*. The sensitivity of one POC-CCA test was much higher than a single Kato-Katz for *S. mansoni* diagnosis before (69.7% versus 28.3%) and after treatment (80.0% versus 4.0%). The POC-CCA test therefore is useful for the diagnosis of *S. mansoni* in preschoolers. It can be used to rapidly identifying *S. mansoni*-infected individuals who need treatment. The application of the POC-CCA test for monitoring of schistosomiasis control interventions needs further investigation.

4.3. Introduction

Recognizing the public health impact of schistosomiasis and soil-transmitted helminth infections, the World Health Organization (WHO) has set a minimum target for the control of morbidity due to these parasitic worm infections, urging members states to regularly treat at least 75% and up to 100%, of all school-aged children at risk of morbidity (WHO, 2002; Savioli et al., 2009). As a result, many African countries have set up national plans of action for the control of schistosomiasis and soil-transmitted helminthiasis, and pursue school-based deworming campaigns (Kabaterine et al., 2006; Fenwick et al., 2009). Experience and lessons from these programs are that they significantly reduce the prevalence and intensity of infection, and thus morbidity (Zhang et al., 2007; Koukounari et al., 2007; Toure et al., 2008a; Knopp et al., 2009).

There is growing evidence that soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and *Schistosoma* infections are acquired already in early childhood (Odogwu et al., 2006; Albonico et al., 2008; Sousa-Figueiredo et al., 2010; Stothard et al., 2011; Dabo et al., 2011; Ekpo et al., 2012). Hence, there is a need for effective and safe treatment of preschool-aged children and their inclusion in preventive chemotherapy is being discussed (Stothard and Gabrielli, 2007; Albonico et al., 2008; Sousa-Figueiredo et al., 2010; Garba et al., 2010). The intensity of infection with soil-transmitted helminths and schistosomes is age-dependent, usually showing a peak in school-aged children and adolescents (Hotez et al., 2006; Tchuente, 2012). For schistosomiasis this might be due to cumulative and increasing water contact behavior of the school-aged child, combined with the

maturation and increasing egg-laying capacity of schistosome worm pairs (Sousa-Figueiredo et al., 2010). Hence, the majority of infected young children might excrete only a few eggs with their feces (for soil-transmitted helminths and *S. mansoni*) and their urine (for *S. haematobium*) (Odogwu et al., 2006; Hotez et al., 2006; Sousa-Figueiredo et al., 2010; Stothard et al., 2011).

It is important to note that the Kato-Katz technique, which is widely used in endemic countries for the diagnosis of *S. mansoni* and soil-transmitted helminths lacks sensitivity, particularly in low endemicity areas, and when infection intensities are low (i.e., in young children or after treatment interventions) (Berhe et al., 2004; Knopp et al., 2008; Knopp et al., 2011a; Knopp et al., 2011b). Hence, improved diagnostic methods for the accurate detection of *S. mansoni* in preschool-aged children, assessment of drug efficacy, and monitoring progress of control programs are desirable. Recent studies have shown that indirect diagnostic tests (e.g., point-of-care circulating cathodic antigen (POC-CCA)) have become valuable alternatives to direct parasitological methods for the diagnosis of *S. mansoni* (Shane et al., 2011; Stothard et al., 2011). Note that the POC-CCA cassette test detects the presence of CCA (a schistosome glycoprotein) in host urine, after being regurgitated into the bloodstream by actively feeding worms, and successive clearance in the host's kidneys. Schistosome antigens can be detected in the serum and urine of infected individuals and their levels are sensitive and specific markers for the presence and intensity of infection (van Dam et al., 1996; Coulibaly et al., 2011; Shane et al., 2011; Stothard et al., 2011). According to the manufacturer, CCA disappear from the urine of patients after 2 and up to 3 weeks after successful treatment. Studies assessing a CCA urine dipstick and a POC-CCA cassette test in preschool-aged children in Uganda and Kenya, respectively, recommended these rapid tests as a useful technique for the detection of *S. mansoni* in that age group (Sousa-Figueiredo et al., 2010; Verani et al., 2011). In our own research, conducted with school-aged children in Côte d'Ivoire, we found that a single urine CCA assay was similarly sensitive as triplicate Kato-Katz thick smears for the diagnosis of *S. mansoni* (Coulibaly et al., 2011). However, the physiological development and biological processes, such as absorption, distribution, metabolism, toxicity and, particularly, excretion are all age and setting dependent (Keiser et al., 2011). Moreover, the effect of geographical variations of *S. mansoni* strains on the performance of POC-CCA cassette test is poorly understood. Hence, there is a need to determine the accuracy of the POC-CCA cassette in preschoolers from different settings as a

diagnostic tool for *S. mansoni*, including its potential as a tool for drug efficacy evaluation, and monitoring of community effectiveness of control interventions.

The current study was designed to assess the accuracy of the commercially available urine POC-CCA cassette test for the diagnosis of *S. mansoni* in preschool-aged children. We designed a 3-week longitudinal study with a treatment intervention, and determined the accuracy of the POC-CCA cassette test before and after the administration of praziquantel.

4.4. Methods

4.4.1. Ethics statement

Our study received ethical clearance from the Ministry of Health and Public Hygiene of Côte d'Ivoire (reference no. 4248/2010/MSHP/CNER). Local authorities in the study area (Azaguié, south Côte d'Ivoire) were informed about the objectives, procedures, and potential risks and benefits of the study. At study onset, a door-to-door information campaign was implemented, and all households in the area informed about the aims and procedures of the study. Written informed consent (or fingerprints of illiterate people) was obtained from parents/guardians of participating preschool-aged children.

Treatment was administered to all preschool-aged children and their mothers, irrespective of their infection status. Participating preschool-aged children were treated with crushed praziquantel tablets at a dose of 40 mg/kg and the efficacy and safety of this intervention have been described elsewhere (Coulibaly et al., 2012). At the end of the study, anthelmintic treatment (single 40 mg/kg oral dose of praziquantel against schistosomiasis, and single 400 mg oral dose of albendazole against soil-transmitted helminthiasis) was offered to all villagers free of charge.

4.4.2. Study area and population

The study, pursuing a 3-week longitudinal design with a treatment intervention and was conducted between August and November 2011 in two villages located in the Azaguié district in south Côte d'Ivoire. The two villages, Azaguié Makouguié (geographical coordinates, 05°37'33" N latitude, 04°09'04" W longitude) and Azaguié M'Bromé (05°39'42" N, 04°08'38" W) are co-endemic for *S. mansoni* and *S. haematobium* (Coulibaly et al., 2011). Subsistence farming is the main economic activity in both villages. Unprotected surface water contact occurs frequently due to the lack of tap water and other sources of clean water. Improved sanitary facilities are the exception rather than the norm. Our door-to-door census

conducted in June 2011 revealed total populations of 931 inhabitants in Azaguié M'Bromé, and 783 people in Azaguié Makouguié. For the current study, emphasis is placed on preschool-aged children aged between 6 years in both villages (n = 367).

4.4.3. *Stool and urine collection*

Using records obtained from the mid-2011 census, a list of all children aged <6 years (considered at preschool-age) was prepared and all of them were invited to participate in our study. Two cross-sectional parasitological surveys were implemented; at baseline and 3 weeks after the administration of praziquantel in order to study the epidemiology of schistosomiasis in preschool-aged children, to assess the efficacy and safety of praziquantel in this age group, and to determine the diagnostic accuracy of the POC-CCA cassette test before and after treatment. Mothers/guardians of participating preschoolers were provided with two plastic containers labeled with unique identification numbers (IDs) at the first day of the respective survey. Mothers/guardians were instructed to collect a morning stool and urine sample of the child, each in one of the two separate containers. After sample collection, the mothers were invited to submit the filled containers until noon to fieldworkers stationed at a central location (the primary school) in each village. Upon submission of the specimens, mothers were handed out a second set of two containers for stool and urine sample collection on the following day.

4.4.4. *Laboratory procedures*

Stool and urine samples were transferred to a nearby laboratory located in the district town Azaguié and processed on the same day. For the diagnosis of *S. mansoni*, duplicate Kato-Katz thick smears were prepared from each stool sample, using 41.7 mg templates (Katz et al., 1972). Kato-Katz thick smears were allowed to clear for at least 30 min before examination under a microscope by experienced laboratory technicians. The number of *S. mansoni* eggs was counted and recorded. Additionally, eggs of soil-transmitted helminths were counted and recorded for each species separately.

For the diagnosis of *S. haematobium*, urine samples were subjected to a filtration method, as described elsewhere (WHO, 2002; Dabo et al., 2011). In brief, 10 ml of vigorously shaken urine were gently pressed through a filter mesh (30 µm; Sefar AG; Heiden, Switzerland) and subsequently a drop of Lugol's iodine solution added to the filter mesh placed on a microscope slide. The filter meshes were then examined quantitatively under a microscope for *S. haematobium* eggs by experienced technicians.

For quality control, 10% of the Kato-Katz and the urine filtration slides were re-examined by a senior technician. In case of disagreement with the initial readings, the results were discussed with the concerned technicians and the slides read a third time until agreement was reached.

Urine samples were additionally subjected to a commercially available POC-CCA cassette test (batch no.: 33112; Rapid Medical Diagnostics, Pretoria, South Africa). The POC-CCA tests were performed as follows: one drop of urine was added to the well of the testing cassette. Once fully absorbed, one drop of buffer (provided with the CCA test kits) was added and the test results were read 20 min after adding the buffer. In case the control bands did not develop, the test was considered invalid and the urine sample was retested with a new POC-CCA cassette. Valid tests were scored as either negative or positive, the latter further stratified into trace, 1+, 2+, or 3+ according to the visibility of the color reaction and manufacturer's instructions. All tests were read independently by two investigators. In case of discordant results, a third independent investigator was consulted, and the results were discussed until agreement was reached (Coulibaly et al., 2011).

Stool and urine samples collected 3 weeks after the administration of praziquantel (single oral dose of 40 mg/kg using crushed tablets) were subjected to the same diagnostic tests as during the pretreatment cross-sectional survey.

4.4.5. *Statistical analysis*

Data were double entered into an Excel spreadsheet, transferred into EpiInfo version 3.2 (Centers for Disease Control and Prevention, Atlanta, United States of America), and cross-checked. In case of discrepancies, the results were traced back to the original data records. Statistical analyses were done using Stata version 10 (Stata Corp., College Station, United States of America). Only children who had complete data records from the baseline surveys (i.e., quadruplicate Kato-Katz thick smears, two POC-CCA cassette tests, and two urine filtrations) were included in the final analysis.

Helminth species-specific fecal egg counts (FECs) as recorded by the microscopists were transformed into numbers of eggs per gram of stool (EPG), multiplying the FEC of each Kato-Katz reading by a factor 24. To assess the infection intensity of each individual, we calculated the arithmetic mean EPG value of quadruplicate Kato-Katz thick smear readings and categorized them according to thresholds given by WHO (WHO, 2002). The three infection intensity classes for *S. mansoni* are (i) light (1-99 EPG); (ii) moderate (100-399

EPG); and (iii) heavy (≥ 400 EPG). Means were compared by Wilcoxon signed rank test and proportions by Pearson's χ^2 test. Based on POC-CCA test scores, the infection intensity of *S. mansoni* was categorized into light (trace or 1+), moderate (2+) and heavy (3+). To investigate the infection intensity of all infected individuals, we calculated the group arithmetic mean of the individual arithmetic mean EPG values. When using the combined results of the POC-CCA tests from days 1 and 2, discordant scores were redefined to provide a single infection intensity measure, as shown in Table 4.1.

For determining the POC-CCA test accuracy, 'trace' results were considered as negative in our 'gold' standard, due to the fact that 'trace' can indicate false positivity. Thus, the accuracy of the Kato-Katz and POC-CCA tests (considering trace results as negative (t-)) for the diagnosis of *S. mansoni* was determined. As diagnostic 'gold' standard before and after treatment we considered the combined results of quadruplicate Kato-Katz thick smears and duplicate CCA(t-), resulting in a positive case as both or either of the tests was positive (see also Midzi et al. (2009) (Midzi et al., 2009)). This assumes an (almost) 100% specificity for the CCA(t-) test. Based on this 'gold' standard, sensitivity, specificity, and negative predictive value (NPV) were calculated. The strength of agreement between quadruplicate Kato-Katz thick smears and the POC-CCA test before treatment was assessed by kappa statistics (κ), as follows: $\kappa = 0$, indicating no agreement; $\kappa = 0-0.2$, indicating poor agreement; $\kappa = 0.21-0.4$, indicating fair agreement; $\kappa = 0.41-0.6$, indicating moderate agreement; $\kappa = 0.61-0.8$ indicating substantial agreement; and $\kappa = 0.81-1.0$, indicating almost perfect agreement (Landis and Koch, 1977; Cohen, 1960). Differences of $p < 0.05$ were considered as statistically significant.

The relationship between POC-CCA cassette test and schistosome infections was assessed as follow: the prevalence of a respective parasite (i.e., *S. mansoni* or *S. haematobium*) based on POC-CCA cassette test results, was used as dependent variable (binary variable: present/absent) and egg counts from each schistosome parasite, expressed as egg per gram (EPG) or egg/10 ml urine was used as explanatory variable. Univariable logistic regression was used to assess the association between the dependent variable (POC-CCA) and each covariate (i.e. EPG or eggs/10 ml urine of each schistosome parasite).

Table 4.1: Scoring scheme to obtain final urine POC-CCA cassette test results.

Day 1 score or <i>vis versa</i>	Day 2 score or <i>vis versa</i>	Final score*
Negative (0)	Trace	Negative (0)
Negative (0)	1+	1+
Negative (0)	2+	1+
Negative (0)	3+	2+
Trace	1+	1+
Trace	2+	1+
Trace	3+	2+
1+	2+	2+
1+	3+	2+
2+	3+	3+

*The final score was determined in case of discordance between test scores from days 1 and 2 and the final score was based on the sum of test scores from both days, divided by two.

4.5. Results

4.5.1. Study adherence and demographic characteristics

Figure 4.1 shows that a total of 367 preschool-aged children were enrolled in the two study villages, 200 girls (54.5%) and 167 boys. Complete parasitological data (i.e., quadruplicate Kato-Katz thick smears, duplicate POC-CCA cassette tests, and duplicate urine filtrations) at the baseline survey before treatment were available for 242 children, 133 from Azaguié M'Bromé and 109 from Azaguié Makouguié. There were 127 girls and 115 boys with a mean age of 3.2 years (range: 2 months to 5.5 years).

Three weeks after the administration of praziquantel, only 86 out of the 242 children had complete parasitological data. There were 43 girls (50.0%) and 43 boys with a mean age of 3.6 years (range: 15 months to 5 years). The two population groups were similar in terms of average age, sex, arithmetic mean FECs of *S. mansoni*, and co-infection status (all $p > 0.05$).

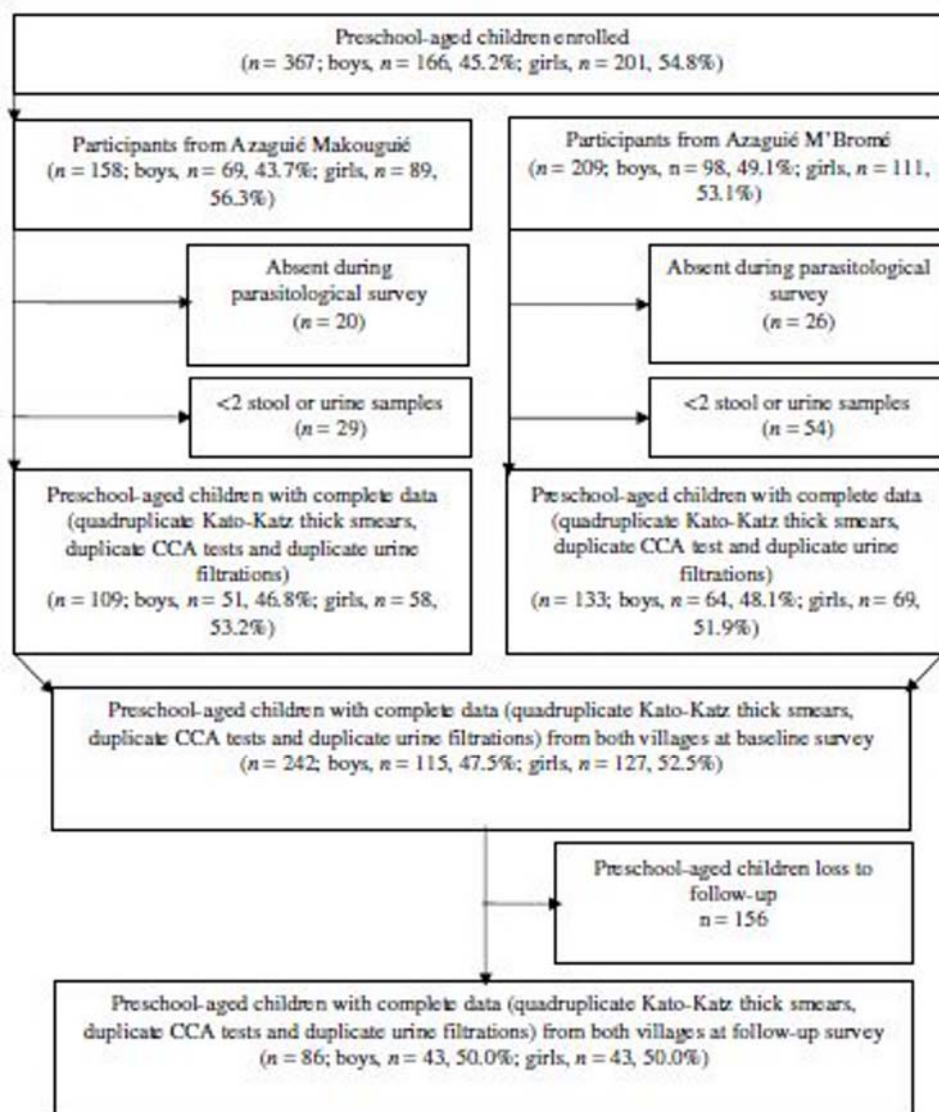


Figure 4.1: Flowchart showing study participation. Flowchart detailing study participation and adherence of preschool-aged children for submitting two stool and two urine samples for the diagnosis of *S. mansoni*, *S. haematobium* and soil-transmitted helminths and before and after administration of praziquantel in the two study villages in the Azaguié district, south Côte d'Ivoire, in August and September 2011.

4.5.2. *S. mansoni* and soil transmitted helminth infections before treatment

Table 4.2 shows the baseline prevalence and intensity of *S. mansoni* infection, as assessed by Kato-Katz and POC-CCA tests. Among the 242 children with complete data records, 56 (23.1%) were found positive for *S. mansoni* by quadruplicate Kato-Katz thick smears. Most infections were of light intensity (n = 40; 71.4%), whereas 12 children (21.4%) had a moderate (100-399 EPG) and four children (7.1%) had a heavy infection (≥ 400 EPG). The group arithmetic mean FEC was 23.4 EPG (95% confidence interval (CI): 13.0-33.7 EPG). A single CCA(t-) test identified 83 children (34.3%) harboring active schistosome infections.

The youngest child infected with *S. mansoni*, as determined by the presence of *S. mansoni* eggs in stool using the Kato-Katz technique, was 8 months. According to the CCA(t-) test results, the earliest infection was observed in a child aged 3 months.

According to quadruplicate Kato-Katz thick smears before treatment, among the 242 preschool-aged children with complete data records, 22 (9.1%), 15 (6.2%) and nine (3.7%) were positive for *T. trichiura*, hookworm and *A. lumbricoides*, respectively Table 4.2. Hookworm and *T. trichiura* infections were exclusively of light intensity ($< 2,000$ EPG and $< 1,000$ EPG, respectively), whereas a third of the *A. lumbricoides* infections were of moderate intensity (5,000-49,999 EPG).

Among the 40 children who were infected with *S. mansoni* according to a single CCA(t-) test, but were negative according to quadruplicate Kato-Katz thick smears, three (7.5%), two (5.0%), and two (5.0%) children were positive for *T. trichiura*, *S. haematobium* and *A. lumbricoides*, respectively. None of these children were infected with hookworm.

4.5.3. *S. haematobium* infection before treatment

Among 242 children at the baseline survey, 26 were infected with *S. haematobium*, giving a prevalence of 10.7% (Table 4.2). Only one child, a 5-year-old girl, had a heavy infection (128 eggs/10 ml of urine). There was no significant association between CCA(t-) results expressed as binary variable (presence/absence of disease) and *S. haematobium* egg counts (OR = 1.2; p = 0.81). Similarly, no significant association was found between CCA(t+) results expressed as binary variable (presence/absence of disease) and *S. haematobium* egg counts (OR = 1.2; p = 0.11).

Table 4.2: Baseline prevalence of *S. mansoni*, *S. haematobium* and soil-transmitted helminths according to diagnostic approach (n = 242).

Parasite	Diagnostic approach	No. of infected individuals	% positive (95% CI)	Light	Moderate	Heavy
				infection intensity (%)	infection intensity (%)	infection intensity (%)
Schistosomiasis						
<i>Schistosoma mansoni</i>	Quadruplicate Kato-Katz thick smears	56	23.1 (17.6-28.3)	39 (86.7)	12 (26.7)	4 (8.9)
<i>Schistosoma mansoni</i> (+ trace)	Single CCA test	156	64.5 (58.4-70.5)	na	na	na
<i>Schistosoma mansoni</i> (- trace)	Single CCA test	83	34.3 (28.3-40.3)	na	na	na
<i>Schistosoma haematobium</i>	Duplicate urine filtration	26	10.7 (6.8-14.7)	18 (94.7)	na	1 (5.3)
Soil-transmitted helminths						
<i>Ascaris lumbricoides</i>	Quadruplicate Kato-Katz thick smears	9	3.7 (1.3-6.1)	6 (66.7)	3 (33.3)	0
<i>Trichuris trichiura</i>	Quadruplicate Kato-Katz thick smears	22	9.1 (1.9-12.7)	22 (100)	0	0
Hookworm	Quadruplicate Kato-Katz thick smears	15	6.2 (3.1-9.3)	15 (100)	0	0

The study was carried out in Azaguié in south Côte d'Ivoire in August and September 2011. Duplicate Kato-Katz thick smears per stool sample and one CCA assay per urine sample were prepared over two consecutive days. Infection intensities are categorized according to thresholds set forth by WHO (WHO, 2002).

na, not applicable

4.5.4. Diagnostic accuracy before treatment

Figure 4.2 shows the correlation between the intensity of *S. mansoni* infection determined by quadruplicate Kato-Katz thick smears, as expressed in EPG, and the CCA(t-) test shown in color scores. We observed a correlation between the color intensity of CCA(t-) test bands and EPG values (odds ratio (OR) = 1.2, p = 0.04).

Comparing the two different methods used for the diagnosis of *S. mansoni*, we found moderate agreement between a single CCA(t-) test and quadruplicate Kato-Katz thick smears ($\kappa = 0.46$, p <0.001; (Table 4.3). The agreement between duplicate CCA(t-) and quadruplicate Kato-Katz thick smears was only fair ($\kappa = 0.36$, p <0.001). Agreement between the two methods was weaker when considering trace results as positive in the urine CCA cassette tests.

According to our ‘gold’ standard, the sensitivity of a single CCA(t-) test (69.7%) was considerably higher than that of a single (28.3%) or quadruplicate Kato-Katz thick smears (47.5%) (Table 4.4). Also the NPV of a single CCA(t-) test (77.4%) was higher than that of a single (59.1%) or quadruplicate Kato-Katz (65.9%). The sensitivity and NPV of a single CCA(t+) test were higher than those of quadruplicate Kato-Katz thick smears and single CCA(t-) (sensitivity: 89.1%; NPV: 84.9%). The specificity of the Kato-Katz technique and CCA(t-) was 100% by definition, whereas the specificity of a single CCA(t+) test was considerably lower (59.3%).

4.5.5. Diagnostic accuracy after treatment

Among the 86 individuals who had complete data records after treatment, *S. mansoni* eggs were detected in Kato-Katz thick smears from 22 (25.6%) individuals during the baseline cross-sectional survey. A single POC-CCA cassette test, considering trace results as negative, revealed 34 preschoolers (39.5%) with an infection. Considering trace results as positive, then a considerably higher number of preschoolers were classified as positive (n = 56, 65.1%).

After treatment, among these 86 children, eggs of *S. mansoni* were only found in two (2.3%) individuals. A single urine CCA(t-) cassette test revealed 20 children (23.3%) with *S. mansoni*, whereas CCA(t+) found 35 (40.7%) infections.

At the 3-week posttreatment evaluation, and considering our ‘gold’ standard (combined results of quadruplicate Kato-Katz thick smears plus duplicate urine CCA(t-) cassette tests), a single CCA(t-) revealed a sensitivity and NPV of 80.0% and 92.4%, respectively (Table 4.4).

Single and even quadruplicate Kato-Katz thick smears showed very low sensitivity (4.0% and 8.0%, respectively) and only moderate NPV (71.8-72.6%).

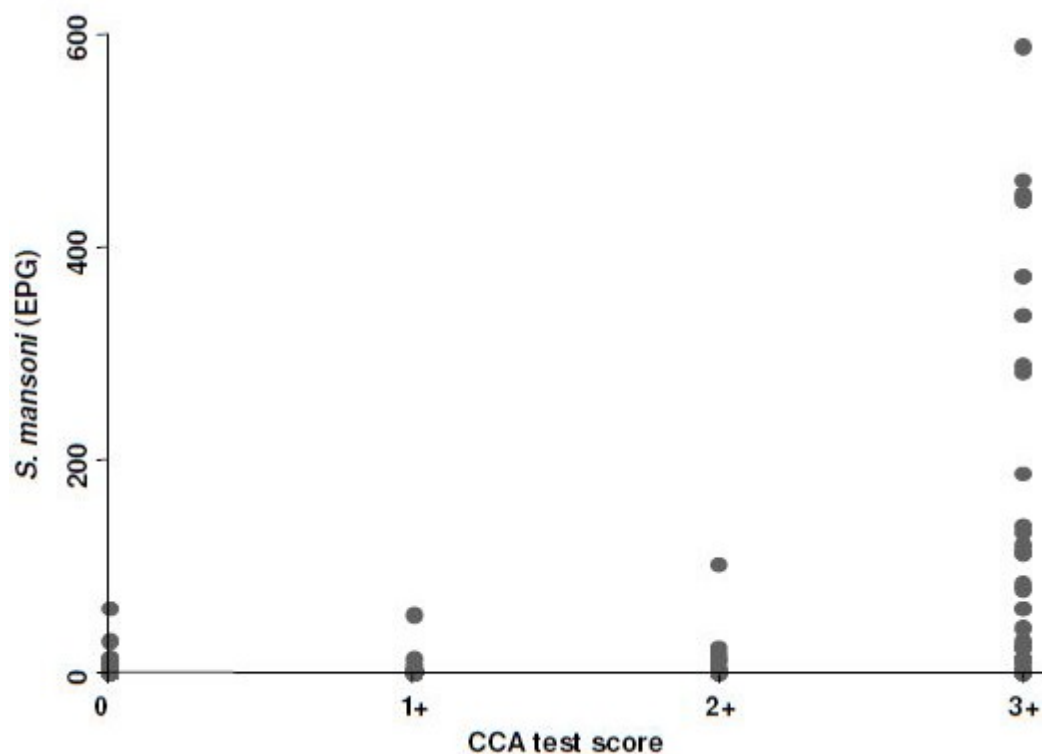


Figure 4.2: Correlation between *S. mansoni* egg counts and CCA test color reaction scores. This figure shows the correlation between the *S. mansoni* eggs per gram of stool (EPG) values, as determined by quadruplicate Kato-Katz thick smears, and a single CCA test with ‘trace’ considered as negative result (negative (0), 1+, 2+ and 3+).

Table 4.3: Agreement between Kato-Katz method and CCA test for the diagnosis of *S. mansoni*.

Diagnostic approach	Test result	Quadruplicate Kato-Katz thick smears			
		Positive	Negative	κ^*	<i>P</i> -value
A single CCA test (+ <i>trace</i>)	Positive	52	104	0.22	p < 0.001
	Negative	5	81		
A single CCA test (- <i>trace</i>)	Positive	43	40	0.46	p < 0.001
	Negative	14	147		

* κ indicating kappa, $\kappa < 0$ indicating no agreement, $\kappa = 0-0.2$ indicating poor agreement, $\kappa = 0.21-0.4$ indicating fair agreement, $\kappa = 0.41-0.6$ indicating moderate agreement, $\kappa = 0.61-0.8$ indicating substantial agreement, and $\kappa = 0.81-1.0$ indicating almost perfect agreement (Cohen, 1960; Landis and Koch, 1977). The study was carried out in Azaguié in south Côte d'Ivoire in August and September 2011.

Table 4.4: Sensitivity, specificity, and negative predictive value (NPV) of different approaches for the diagnosis of *S. mansoni*.

	Before treatment			After treatment		
	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)
Combined results as ‘gold’ standard*						
One Kato-Katz thick smear	28.3 (20.5-37.3)	100 (97.0-100)	59.1 (52.1-65.9)	4.0 (0.1-20.4)	100 (94.1-100)	71.8 (61.0-81.0)
Two Kato-Katz thick smears	36.1 (27.5-45.4)	100 (97.0-100)	61.8 (54.7-68.6)	4.0 (0.1-20.4)	100 (94.1-100)	71.8 (61.0-81.0)
Three Kato-Katz thick smears	42.9 (33.8-52.3)	100 (97.0-100)	64.4 (57.2-71.2)	4.0 (0.1-20.4)	100 (94.1-100)	71.8 (61.0-81.0)
Four Kato-Katz thick smears	47.5 (38.3-56.8)	100 (97.0-100)	65.9 (58.6-72.7)	8.0 (0.9-26.0)	100 (94.1-100)	72.6 (61.8-81.8)
One POC-CCA (<i>incl trace</i>)	89.1 (81.2-93.5)	59.3 (50.1-68.1)	84.9 (75.5-91.7)	84.0 (63.9-95.5)	77.0 (64.5-86.8)	92.2 (81.1-97.8)
Two POC-CCA (<i>incl trace</i>)	97.5 (92.8-99.5)	99.5 (90.1-100)	93.0 (83.0-98.1)	100 (86.3-100)	96.6 (93.1-100)	100 (90.3-100)
One POC-CCA (<i>excl trace</i>)	69.7 (60.7-77.8)	100 (97.0-100)	77.4 (70.1-83.6)	80.0 (59.3-93.2)	100 (94.1-100)	92.4 (83.2-97.5)
Two POC-CCA (<i>excl trace</i>)	91.6 (85.1-95.9)	100 (97.0-100)	92.5 (86.6-96.3)	96.0 (79.6-99.9)	100 (94.1-100)	98.4 (91.3-100)
Combined results as ‘gold’ standard**						
One Kato-Katz thick smear	18.1 (12.9-24.3)	100 (93.4-100)	26.0 (20.1-32.5)	2.0 (0.1-10.6)	100 (90.3-100)	42.4 (31.7-53.6)
Two Kato-Katz thick smears	22.9 (17.1-29.5)	100 (93.4-100)	27.1 (21.1-33.9)	2.0 (0.1-10.6)	100 (90.3-100)	42.4 (31.7-53.6)
Three Kato-Katz thick smears	27.1 (20.9-34.1)	100 (93.4-100)	28.3 (22.0-35.2)	2.0 (0.1-10.6)	100 (90.3-100)	42.4 (31.7-53.6)
Four Kato-Katz thick smears	29.8 (23.4-36.9)	100 (93.4-100)	29.0 (22.6-36.1)	4.0 (0.5-13.7)	100 (90.3-100)	42.9 (32.1-54.1)
One POC-CCA (<i>incl trace</i>)	83.0 (76.8-88.1)	100 (93.4-100)	62.8 (51.7-73.0)	82.0 (68.6-91.4)	100 (90.3-100)	70.6 (50.6-85.3)
Two POC-CCA (<i>incl trace</i>)	98.4 (95.4-99.7)	100 (93.4-100)	94.7 (85.4-98.9)	100 (92.9-100)	100 (90.3-100)	100 (90.3-100)
One POC-CCA (<i>excl trace</i>)	44.1 (36.9-51.6)	100 (93.4-100)	34.0 (26.6-41.9)	40.0 (26.4-54.8)	100 (90.3-100)	54.5 (41.8-66.9)
Two POC-CCA (<i>excl trace</i>)	58.0 (50.6-65.1)	100 (93.4-100)	40.6 (32.2-49.5)	48.0 (33.7-62.6)	100 (90.3-100)	58.1 (44.8-70.5)

* Combined results of quadruplicate Kato-Katz thick smears and duplicate CCA tests with “trace” as negative

** Combined results of quadruplicate Kato-Katz thick smears and duplicate CCA tests with “trace” as positive

The study was carried out in Azaguié in south Côte d’Ivoire in August and September 2011.

4.5.6. *Day-to-day variability of POC-CCA cassette test scores*

Table 4.5 shows the day-to-day variability of the POC-CCA cassette test scores before (n = 242) and 3 weeks after the administration of praziquantel (n = 86). At baseline 156 (64.5%) and 145 (59.9%) were found CCA positive on day 1 and day 2, respectively. After treatment, 35 (40.7%) children on day 1 and 32 (37.2%) children on day 2 showed a positive POC-CCA test. Comparing POC-CCA cassette test results from both days, revealed no statistically significant difference in test results before (p = 0.619) and after (p = 0.756) treatment.

There was relatively little day-to-day variation, both before and after treatment. For example, before treatment, about half of the paired POC-CCA test results showed the same scores, whereas 127 (52.5%) children had discordant scores, with the highest discrepancy observed between negative and trace results. Considering trace results as negative, the percentage of discordant results decreased to 22.7%. In the posttreatment survey, none of the children with duplicate CCA cassette tests performed showed 3+ scores on both days. Discordant POC-CCA test scores between days 1 and 2 were found in slightly more than half of the children (n = 44, 51.2%) with the highest number of discordant results between negative and trace results. The concordance between POC-CCA cassette test scores from days 1 and 2 increased with infection intensity (based on POC-CCA cassette test band color), both before and after treatment

Among those 86 preschool-aged children who had complete data records before and after treatment, and considering the higher of the two color reactions in the duplicate CCA tests as the final score showed that the number of tests scored 3+ before treatment decreased by 76.5% following treatment. A decrease of 22.5% of POC-CCA tests scored as trace was observed 3 weeks posttreatment. Among seven preschool-aged children scored as trace-positive before treatment, four became CCA-negative following treatment, whereas the remaining three were diagnosed CCA-positive (two children with 1+ and one child with 2+). Nine (16.1%) children among the 56 children detected with CCA (trace included) had unchanged test scores after treatment. The number of children found CCA-negative increased sharply 3 weeks after a single dose of praziquantel, with a particularly steep decrease of heavy infections ($\chi^2 = 6.50$, p = 0.011) (Figure 4.3).

Based on our cohort of 86 children, when considering trace results as positive at the posttreatment follow-up, 32 children were CCA positive. Among them 23 children were egg-negative at the baseline survey and 9 children had baseline FECs ranging between 6 and 450

EPG. However, when considering trace results as negative only 13 children were still CCA positive at follow-up. Among them 10 children were egg-negative at baseline and 3 children had baseline FECs ranging between 132 and 588 EPG. Whatever the status (positive or negative) given to trace results, more than three-quarter of children found CCA-positive at follow-up were egg-negative at the baseline survey.

Table 4.5: Number of preschool-aged children failing in each CCA test score before and after treatment, stratified by urine collection day (n = 86).

POC-CCA cassette test score	Before treatment (<i>n</i> = 242)					After treatment (<i>n</i> = 86)				
	Negative (0)	Trace	1+	2+	3+	Negative (0)	Trace	1+	2+	3+
Day 1	86	73	40	14	29	51	15	10	9	1
Day 2	97	65	28	23	29	54	17	9	3	3
Combined scores (days 1 and 2) ^a	105	28	50	27	32	59	3	15	7	2
Higher score (either day 1 or day 2) ^b	57	76	47	23	39	35	31	8	8	4

n = 86, Day 1: first day of urine collection, Day 2: second day of urine collection

^a Combined POC-CCA cassette test (days 1 and 2), as defined in Table 1

^b The higher POC-CCA cassette test score from either day 1 or day 2 was considered as final score

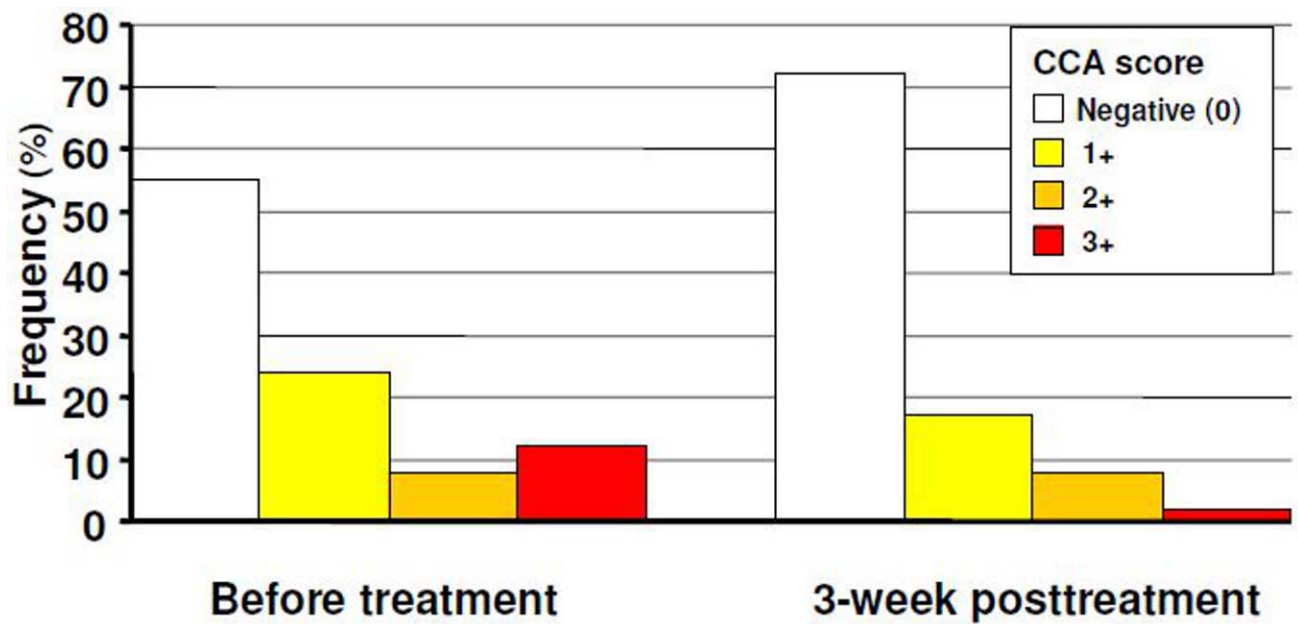


Figure 4.3: Frequency of CCA test scores before (n = 242) and after praziquantel administration (n = 86). The frequency of the CCA test score (0, 1+, 2+, and 3+) before and after treatment with praziquantel was determined based on combined score from days 1 and 2, as show in Table 4.1. Note that trace results were considered as negative.

4.5.7. *Test requirements of POC-CCA cassette and Kato-Katz*

Table 4.6 summarizes key test requirements and compares them between Kato-Katz (standard test) and POC-CCA (newly developed test) for the diagnosis of *S. mansoni*. Important test requirements include the ease of obtaining and analyzing the samples, cost considerations and diagnostic accuracy.

Table 4.6: Comparison of test requirements of POC-CCA cassette and Kato-Katz thick smear.

Test requirement	POC-CCA cassette test	Kato-Katz method
Sample	Urine	Stool
Stage of worm detected	Immature and adult worms through antigens	Adult worms through eggs
Number of sample needed for accurate diagnosis	One sample, even in low endemicity setting	Several samples, especially in low endemicity setting
Sample collection	Straightforward	Difficult, reluctance to provide stool, especially among adults
Time spend to obtain test result at the laboratory	25 min	Several hours
Skill of the person who perform the test	Non-specialized personnel	Specialized personnel
Logistic	Car for transport, POC-CCA test kit	Car for transport, Kato-Katz kit, microscope, microscope slide, electricity

Not detailed requirements of each test were mentioned in this table, but only the main requirements of each test

4.6. Discussion

There is growing awareness that in high endemicity settings, schistosomiasis is also common in preschool-aged children, and hence these young children might need to be included in deworming campaigns (Albonico et al., 2008; Sousa-Figueiredo et al., 2010). The Kato-Katz technique has been the backbone of intestinal schistosomiasis (and soil-transmitted helminthiasis) diagnosis in epidemiological studies for decades. However, it shows a low sensitivity for detecting low-intensity infections, which are commonly seen in young children and in communities undergoing regular treatment (Lin et al., 2008; Knopp et al., 2011a; Verani et al., 2011). Recent studies have shown that the urine-based CCA test is a promising method for the diagnosis of *S. mansoni* in preschoolers and school-aged children (Stothard et al., 2006; Coulibaly et al., 2011; Shane et al., 2011; Stothard et al., 2011; Verani et al., 2011).

In the present work, we investigated the accuracy of this POC-CCA cassette test in preschool-aged children from south Côte d'Ivoire before and after administration of a single oral dose of praziquantel (40 mg/kg) and compared its performance to that of multiple Kato-Katz thick smears.

We found that a single POC-CCA is more sensitive than quadruplicate Kato-Katz thick smears before and 3 weeks after praziquantel treatment. The intensity of a positive CCA test band reaction was significantly correlated with the *S. mansoni* egg burden quantified by the Kato-Katz technique. There was a sharp decrease of CCA tests scored 3+ after treatment and an increase in tests scored negative or trace. Most of the children who were CCA-positive in the posttreatment follow-up survey were either egg-negative at baseline or heavily infected. The youngest child identified as infected with *S. mansoni* applying the POC-CCA cassette test was 3 months old. Eggs in stool examined with the Kato-Katz method were only detected in children aged 8 months and above.

Our results corroborate recent findings from Kenya and Uganda, where CCA tests detected *S. mansoni* infections in preschool-aged children considerable earlier and at higher frequency than the Kato-Katz technique and an enzyme-linked immunosorbant assay (ELISA) kit to test for host antibodies to soluble egg antigens (Stothard et al., 2011; Verani et al., 2011). The results reported here also extend on our own recent observations in the same study

area and that of other groups made elsewhere that a commercially available urine CCA test shows a considerably higher sensitivity than the widely used Kato-Katz technique for the diagnosis of *S. mansoni* in school-aged children (Coulibaly et al., 2011; Shane et al., 2011).

As confirmed in the present study, the prevalence and intensity of *Schistosoma* infections in preschool-aged children is rather low (Odogwu et al., 2006; Sousa-Figueiredo et al., 2010; Keiser et al., 2011; Stothard et al., 2011). Hence, the Kato-Katz and other direct diagnostic methods have limitations when it comes to accurate individual diagnosis. Moreover, the consistency of stools in very young children is mostly diarrheic what renders the preparation of Kato-Katz thick smears difficult, which further challenges an accurate diagnosis. The constraints of using diarrheic stool as well as stool of breastfed infants for helminth diagnosis has been reported elsewhere (Teesdale et al., 1985; Goodman et al., 2007). In that respect, one needs to consider that in the humid tropics, viral, bacterial, and multi-parasitic infections causing diarrhea are very common (Haque et al., 2003; Thapar and Sanderson, 2004), and that preschool-aged children are particularly prone to such infections (Annan et al., 1986; Thapar and Sanderson, 2004). Hence, the Kato-Katz technique has shortcomings for helminth diagnosis in this age-group.

The implementation of large-scale schistosomiasis control programs that are based on preventive chemotherapy reduces the prevalence and, most importantly, the intensity of *Schistosoma* infections (Toure et al., 2008b; Fenwick et al., 2009; Zhou et al., 2011). Hence, the endemicity is lowered, which goes hand-in-hand with a reduced accuracy of the Kato-Katz technique (Bergquist et al., 2009; Knopp et al., 2011b). In view of recent discussions regarding schistosomiasis elimination (Rollison et al., 2012), the need for highly sensitive and specific diagnostic tool for the diagnosis of *S. mansoni* and other *Schistosoma* species after extensive preventive chemotherapy campaigns and additional interventions cannot be emphasized enough. However, some weaknesses seem to go against the use of POC-CCA as a diagnostic tool for control programs. First, the Kato-Katz method allows for diagnosis of other helminth infections (e.g., soil-transmitted helminthiasis), which commonly co-exist where schistosomiasis is endemic. Second, the Kato-Katz technique provides a quantitative measure to the infections, which guide the control program interventions. Third, the cost of a single POC-CCA cassette (approximately US\$ 2.0) is somewhat higher than the total costs of performing a single Kato-Katz thick smear in epidemiological surveys (US\$ 1.7) (Speich et al., 2012). Hence, the costs for individual diagnosis currently limit the use and attractiveness

for program managers for larger scale applications. For individual diagnosis, however, it should be noted that the costs largely dependent on the patient's economical situation.

Our finding of very young children diagnosed with *S. mansoni* when using the urine POC-CCA cassette test (3 months old), and only 5 months later when using the Kato-Katz technique raises an alarm bell. Current control programs focus on the school-aged population (usually starting at an age of 5-6 years), and hence a considerable number of infected children might be restrained from treatment for perhaps 3-4 years. Recent studies discussed the potential impact of early infections that remain untreated for several years on child health due to the cumulative effect of repeated infections (Balen et al., 2006; Chiavaroli and Grima, 2008; Andrade, 2009; Shane et al., 2011). Our observations are also important from a surveillance point of view. Indeed, first the POC-CCA test revealed the age of first *S. mansoni* infection several months earlier than the Kato-Katz technique and, second, we found that three quarter of the people who were CCA-positive at follow-up were egg-negative at baseline. It seems that these children were infected with immature worms that praziquantel was not able to kill. Hence, despite the aforementioned limits of the POC-CCA cassette test, some advantages deserve to be highlighted. First, POC-CCA is based on simple-to-use urine test, which can be performed by non-specialized personnel. Second, POC-CCA does not depend on electricity, and hence can be utilized in remote rural areas that are currently not connected to the power grid. Third, collection of urine samples for POC-CCA is more straightforward and less invasive than collection of stool for Kato-Katz thick smears. Hence, the time spent from the field (sample collection; urine for POC-CCA cassette test versus stool for Kato-Katz thick smears) to the laboratory (implementation; at least 25 min for POC-CCA cassette test versus several hours for Kato-Katz thick smears) places the POC-CCA in a favorable position. Fourth, a POC-CCA test is able to detect prepatent infections, whereas the Kato-Katz technique can only detect patent infections. Fifth, hundreds of patients could be tested by POC-CCA per day, whereas Kato-Katz is more time consuming. Numberand sixth, simplification of logistics in field settings (Table 4.6). Taken together, the POC-CCA cassette test is an adequate and most useful tool for rapid identification of infected individuals and high-risk communities that warrant interventions at the individual patient level and at the community level with the goal to lower morbidity and transmission of schistosomiasis. Efforts might thus be warranted by the United Nations through its agencies to allow extension of the use of POC-CCA tests in schistosome-endemic areas where financial resources are often limited.

Our study shows that the number of positives determined by the POC-CCA test after treatment is considerably higher than that revealed by the Kato-Katz technique. Indeed, the Kato-Katz technique found a very low prevalence after treatment (2.3%), whereas POC-CCA test results revealed several-fold higher prevalences (23.3% considering trace results as negative and 40.7% considering trace results as positive). These differences might be explained by the following reasons. First, the Kato-Katz technique is underestimating the prevalence due to very low infection intensities after treatment (Wilson et al., 2006). Second, in the current study, Kato-Katz thick smears were read shortly after slide preparation (within 30-60 min). Prompt microscopic examination of Kato-Katz thick smears is recommended for the concurrent diagnosis of soil-transmitted helminths, particularly hookworm (Knopp et al., 2008), but the optimal detection of *S. mansoni* eggs is after clearing the slides for 24 hours (Deelder et al., 2011). On the other hand, the POC-CCA test might overestimate the prevalence (i.e., in case CCA is still excreted in urine more than 3 weeks after treatment despite the death of adult worms). Studies conducted to date are inconclusive on when exactly CCA is eliminated from urine below detection limits (van Dam et al., 1996; Nibbeling et al., 1998; Legesse and Erko, 2007). In view of the aforementioned limitations of our direct parasitological approach, it is conceivable that CCA-positive, egg-negative cases are false-negatives based on the Kato-Katz technique (Midzi et al., 2009).

Assessing the converting proportion of POC-CCA test color band scores after treatment, we observed a significant increase of negative scores and decrease of trace and 3+ scores, despite only considering the combined score per individual over two test days. Since we also found that the FECs detected with the Kato-Katz method correlate with the test band color intensity, the POC-CCA test might indeed reveal formerly heavily infected individuals as still positive. In light of the absence of a real ‘gold’ standard in our study, future investigations using highly sensitive and specific diagnostic methods (i.e., a polymerase chain reaction (PCR) (Pontes et al., 2002), or detection of circulating anodic antigen (CAA) by an up-converting phosphor technology (UPT)-based lateral flow (LF) assay (Corstjens et al., 2008)) are of need to investigate the true accuracy of a urine CCA cassette test after treatment, and hence its applicability for monitoring the success of schistosomiasis control programs.

4.7. Conclusion

In conclusion, a single POC-CCA urine cassette test appears to be more sensitive than multiple Kato-Katz thick smears for the diagnosis of *S. mansoni* in preschool-aged children. It

is therefore an appropriate tool for the rapid identification of *S. mansoni*-infected individuals, including preschool-aged children, and of high-risk communities before the onset of control interventions. Its applicability to accurately assess infections a few weeks after praziquantel administration needs further investigation and comparison with highly sensitive and specific diagnostic tools.

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5. Epidemiology of schistosomiasis in two high-risk communities of south Côte d'Ivoire with particular emphasis on pre-school-aged children

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5.1. Abstract

Schistosomiasis control efforts mainly target school-aged children. We studied the epidemiology of schistosomiasis in two high-risk communities in south Côte d'Ivoire, placing particular emphasis on pre-school-aged children. We used a suite of diagnostic techniques, including Kato-Katz, urine filtration, reagent strips, and urine circulating cathodic antigen cassettes. Risk factors for schistosomiasis were determined by focus group discussions and a structured questionnaire. The prevalence of *Schistosoma mansoni* in the two study villages among the pre-school-aged children was 20.9% and 25.0%, whereas several-fold higher prevalences were found in school-aged children (58.7-68.4%) and adolescents/adults (59.5-61.7%). The prevalences of *S. haematobium* in the three age groups were 5.9-17.3%, 10.9-18.4% and 3.8-21.3%, respectively. Most participants had light-intensity infections. Mothers' occupations and older siblings play important roles in the epidemiology of schistosomiasis in pre-schoolers. In the current epidemiologic settings, more attention is warranted on pre-school-aged children and adolescents/adults for successful schistosomiasis control.

5.2. Introduction

Schistosomiasis remains of considerable public health importance in sub-Saharan Africa (Southgate et al., 2005; Hotez et al., 2006; Steinmann et al., 2006; Utzinger et al., 2009) It is widely acknowledged that schistosomiasis infection prevalence and intensity curves show peaks in children aged 6-15 years, after which prevalence and intensities decline gradually with age (Sleigh et al., 1985; Woolhouse, 1998) Hence, the current global strategy emphasizes preventive chemotherapy (i.e., regular administration of the anthelmintic drug praziquantel), which is primarily targeted to school-aged children. Conversely, pre-school-aged children (<6 years) and adolescents/adults (16 years and above) are often neglected from preventive chemotherapy. Justification for the exclusion of the latter age group is given by the lower frequency of water contact compared to school-aged children and the development of an acquired protective immunity against schistosomiasis (Wilkins et al., 1984; Butterworth et al., 1985; Verani et al., 2011) With regard to pre-school-aged children, they are thought to be at low risk of schistosomiasis due to infrequent contact with freshwater bodies. Moreover, there is a paucity of pharmacokinetic data, including safety, of praziquantel in the pre-school-aged child population (Allen et al., 2002; Geary et al., 2010), and the current lack of an appropriate formulation (e.g., syrup) of praziquantel for young children (Garba et al., 2010)

are important barriers for the inclusion of pre-school-aged children in preventive chemotherapy campaigns.

However, there is growing recognition that in areas of high endemicity, pre-schoolers are at considerable risk of schistosomiasis (Bosompem et al., 2004; Garba et al., 2010; Dabo et al., 2011) Hence, discussions are underway whether pre-school-aged children should be included in preventive chemotherapy campaigns, at least in highly endemic. Recent studies carried out in the Azaguié district, south Côte d'Ivoire, revealed that both *Schistosoma mansoni* and *S. haematobium* co-exist, and particularly high levels of co-infections were found in the villages of Azaguié Makouguié and Azaguié M'Bromé (N'Guessan et al., 2006; Coulibaly et al., 2011) However, the epidemiology of schistosomiasis and risk factors for early childhood infection have not been investigated in this part of Côte d'Ivoire. To fill this gap, we designed a cross-sectional parasitologic and questionnaire survey to determine the prevalence and intensity of *S. mansoni* and *S. haematobium* infection in pre-school-aged children, including risk factors for early child infection. For comparison, we also screened older individuals for schistosome infections. Our findings are discussed in the frame of integrated schistosomiasis control and indeed new efforts that aim at elimination of schistosomiasis (Rollinson et al., 2012)

5.3. Materials and methods

5.3.1. Ethical consideration and treatment

Ethical clearance was obtained from the Ministry of Health and Public Hygiene of Côte d'Ivoire (reference no. 4248/2010/MSHP/CNER). Village authorities were informed and, after they had agreed, the objectives, procedures, and potential risks and benefits of the study were explained to the heads of households by three of us (JTC, YKN, and EKN). Written informed consent or fingerprints (of illiterate people) were obtained from participants aged 16 years and above, and from parents or legal guardians on behalf of children (<16 years).

At the end of the study, free anthelmintic treatment was offered to the whole population in both villages (i.e., praziquantel, 40 mg/kg of body weight using a dose-pole against schistosomiasis; albendazole, 400 mg, against soil-transmitted helminthiasis). For children aged <6 years, praziquantel was given according to their weight, using crushed 600 mg tablets, with results on the efficacy and safety of crushed praziquantel tablets reported elsewhere (Coulibaly et al., 2012)

5.3.2. *Study area and population*

The study was carried out between June and September 2011 in two villages, Azaguié Makouguié (geographic co-ordinates, 05°37'33" N latitude and 04°09'04" W longitude) and Azaguié M'Bromé (05°39'42" N, 04°08'38" W), both located in the district of Azaguié, south Côte d'Ivoire. Subsistence farming is the main economic activity in both villages. Many households lack access to permanent clean water and open defecation is frequently practiced. *S. mansoni* and *S. haematobium* are co-endemic in this area (Coulibaly et al., 2011).

5.3.3. *Population census and sample size calculation*

A detailed census was carried out in June 2011 as follows. After discussions with village authorities, four community members in each of the two villages were trained to conduct the census. All households were visited and socio-demographic characteristics were collected (e.g., name, age, sex, relationship with household head, and main activity of each individual). The primary source for obtaining the children's age was their birth certificate. In case birth certificates were not available, we checked the children's vaccination card, which includes the birth date. The age of children without any of these two official documents, was declared by the mothers. Unique identifiers (IDs) were attached to all households and the individual inhabitants. As a household, we considered a structure where people regularly share their food (most commonly, this was a male household head, a women, and the parents' children). The village census revealed 931 and 783 individuals in Azaguié M'Bromé and Azaguié Makouguié, respectively. Among them, there were 209 children below the age of 6 years in Azaguié M'Bromé and 158 in Azaguié Makouguié.

Sample size calculation for pre-school-aged children was done as follows. First, we assumed a prevalence of *Schistosoma* (either *S. mansoni* or *S. haematobium*) of 20% among pre-school-aged children (i.e., approximately a quarter of the 80% *S. mansoni* infection prevalence observed in school-aged children in Azaguié in 2010).(Coulibaly et al., 2011) Second, we allowed for a relatively low compliance rate (70%) due to the difficulty of obtaining stool and urine samples from pre-school-aged children. Third, we considered an alpha error of 5%. Thus, we needed approximately 300 pre-school-aged children. Since the total number of pre-school-aged children in the two villages was only slightly higher, we decided to invite all of them. For the sample size in the older age group (≥ 6 years), a quarter of the total population was selected in each village (drawing every 4th household member

using random number lists). Whenever the selection fell onto a pre-school-aged child, we selected the next individual until we reached the required sample size.

5.3.4. *Field procedures*

The geographic coordinates of all households where at least one pre-school-aged child was registered and water contact points were recorded, using a hand-held global positioning system (GPS) device (Garmin GPS map 62 ST).

Pre-school-aged children were asked to provide two stool and two urine samples over consecutive days. The day before sample collection, children's mothers/guardians were given two empty containers, one for stool and the other for urine collection. Filled containers (small portion of stool and at least 10 ml of urine) were collected and labeled with unique IDs, and mothers/guardians were issued with new empty containers for sample collection the next day. Given the difficulty to collect biological samples in this age group, mothers/guardians were allowed to obtain samples from their young children at any time of the day (usually in the early morning hours). In order to have comparable data, stool and urine samples from older participants (school-aged children, adolescents, and adults) were also collected in the morning hours.

5.3.5. *Laboratory procedures*

Stool and urine samples were transferred to a laboratory in the Azaguié health center. Diagnostic work-up was completed on the day of sample collection. (Coulibaly et al., 2011; Coulibaly et al., 2012) Duplicate Kato-Katz thick smears, using 41.7 mg templates (Katz et al., 1972) were prepared on microscope slides from each stool sample. Hence, our diagnostic approach consisted of quadruplicate Kato-Katz thick smears (two stool samples, each subjected to duplicate Kato-Katz). The slides were allowed to clear for at least 30 min before microscopic examination for eggs of *S. mansoni* and soil-transmitted helminths by an experienced laboratory technician.

Four hundred eighty-eight urine samples were examined as follows. First, the samples were visually inspected and classified into clear, cloudy, and bloody (macrohematuria). Second, microhematuria was determined using reagent strips (Combur-test®; Roche Diagnostics, Basel, Switzerland), following a semi-quantitative assessment scheme: negative, 1+ (approximately 5-10 erythrocytes/ μ l of urine), 2+ (approximately 25 erythrocytes/ μ l of urine), 3+ (approximately 50 erythrocytes/ μ l of urine), and 4+ (approximately 250

erythrocytes/ μ l of urine). Third, urine samples were subjected to a circulating cathodic antigen (CCA) cassette test (Rapid Medical; Pretoria, South Africa) for *S. mansoni* diagnosis (Coulibaly et al., 2011). Finally, samples were subjected to a filtration method. In brief, samples were vigorously shaken, 10 ml filtered using small-sized filters (aperture 20 μ m; Sefar, Heiden, Switzerland), placed on a slide, and *S. haematobium* eggs enumerated under a microscope by an experienced laboratory technician.

5.3.6. *Questionnaire survey and focus group discussion (FGDs)*

A questionnaire was administered to mothers/guardians of pre-school-aged children to determine risk factors for schistosomiasis. Prior to administration, our questionnaire was pre-tested with 6 women not otherwise involved in the study. The interviews were conducted in the local language (Abbey) by trained enumerators. Mothers/guardians' education and profession, knowledge about schistosomiasis, recent history of migration, personal hygiene, common playing and recreational activities, placing particular emphasis on water activities of their children, and access to health care were determined.

Subsequently, in each village, based on the parasitological data, one FGD was done with mothers/guardians of Schistosoma-infected pre-school-aged children and a second FGD with mothers/guardians of non-infected children. The FGDs were built around occupational, bathing, washing, cooking, and recreational behavior of the mothers/guardians and the children they care for.

5.3.7. *Statistical analysis*

Parasitological and questionnaire data were entered twice into an Excel spreadsheet, transferred onto EpiInfo 3.2 (Centers for Disease Control and Prevention; Atlanta, GA) and cross-checked. All analyses were carried out in STATA version 10 (Stata Corp.; College Station, TX). Participants with complete parasitologic data (i.e., quadruplicate Kato-Katz thick smears and duplicate urine filtration for all participants, and an additional two urine CCA tests and two reagent strips (for microhematuria) for pre-school-aged children) were included in the final analysis. Means and proportions of interest were calculated and comparisons done using Kruskal-Wallis and Pearson's χ^2 tests.

The intensity of *S. mansoni* was expressed as eggs per gram of stool (EPG) and categorized into light (1-99 EPG), moderate (100-399 EPG), and heavy infections (\geq 400

EPG). The intensity of *S. haematobium* infection was grouped into light (1-49 eggs/10 ml of urine) and heavy infections (≥ 50 eggs/10 ml of urine).

We employed a logistic regression model to assess significant associations between a *Schistosoma* infection and sex, age, village, and mothers/guardians behavioral factors. For both *Schistosoma* species, a baseline model was established with infected pre-school-aged children defined as cases. Sex (as binary variable), age (as categorical variable), village (as binary variable), and mothers/guardians behavioral factors (as binary or categorical variable) were incorporated into the model. A backward elimination approach was used, and non-significant associations ($p > 0.2$) were removed, one at a time. Adjusted odds ratios (OR), including 95% confidence intervals (CI) were calculated.

In order to establish village-specific schistosomiasis risk maps for preschoolers, the geographic coordinates of each household inhabited by a child younger than 6 years and the water contact points indicated by the mothers/guardians were transformed into a universal transverse mercator (UTM) system, and transferred into ArcView 3.2 (ESRI, Redlands, CA). Pre-school-aged children were stratified into schistosome-free, single species infection with either *S. mansoni* or *S. haematobium*, or co-infection with both schistosome species.

5.4. Results

5.4.1. Study adherence

Figure 5.1 shows that a total of 783 individuals were selected from both villages, 429 (54.8%) in Azaguié M'Bromé and 354 in Azaguié Makouguié. In Azaguié M'Bromé, there were 209 (48.7%) pre-school-aged children and 220 participants aged 6 years and above. In Azaguié Makouguié, there were 158 (44.6%) pre-schoolers and 196 older participants. Overall, 242 and 259 individuals had complete parasitologic data (i.e., quadruplicate Kato-Katz thick smears and two urine filtrations and, for pre-school-aged children additionally two CCA cassette tests and two reagent strips) in Azaguié Makouguié and in Azaguié M'Bromé, respectively.

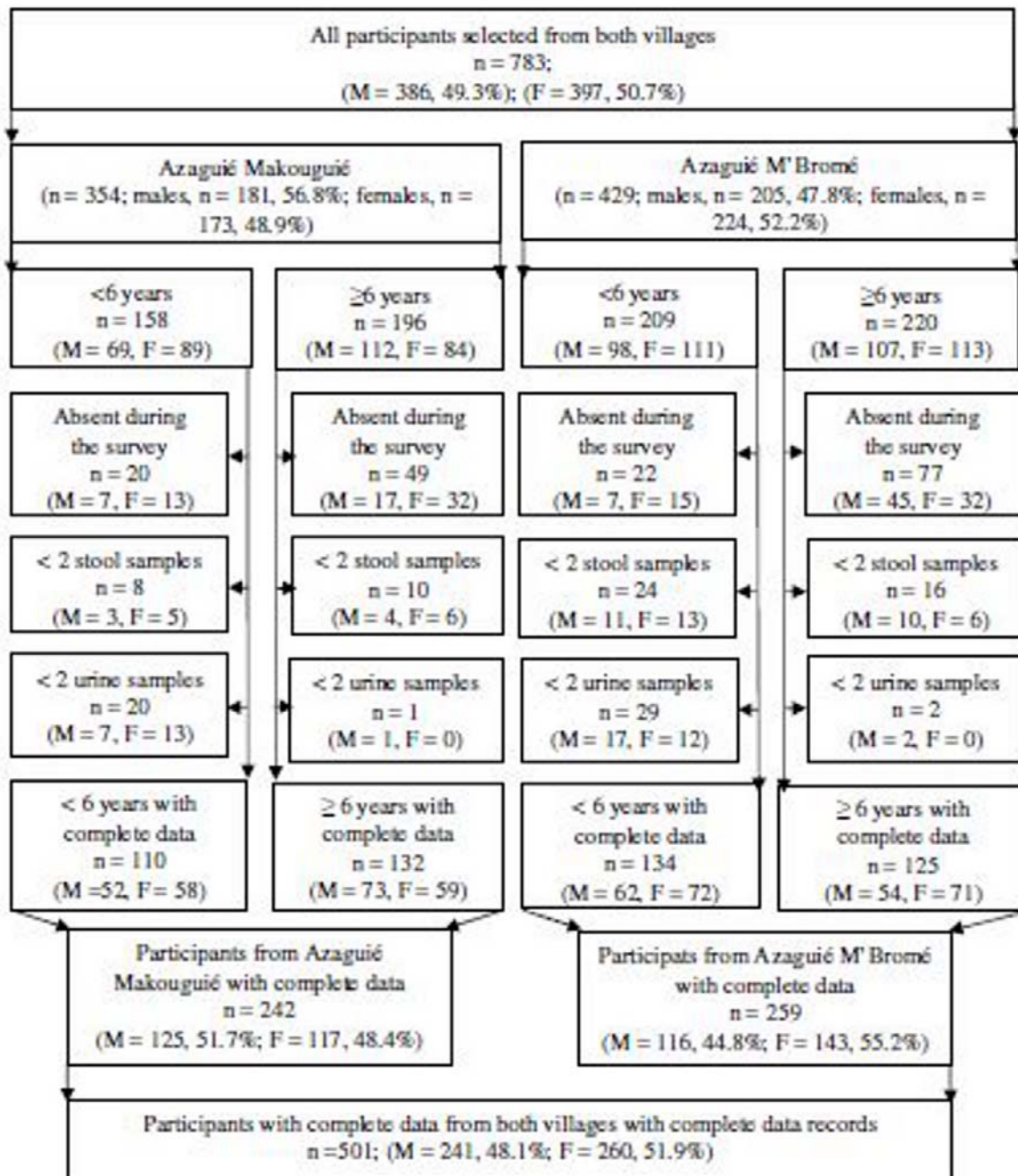


Figure 5.1: Flowchart detailing the study participation and adherence of pre-school-aged children for submission of two stool and two urine samples for the diagnosis of *S. mansoni* and *S. haematobium* in Azaguié, south Côte d'Ivoire, in mid-2011.

5.4.2. *Demographic characteristics*

Table 5.1 summarizes the demographic characteristics of the study population, stratified by pre-school-aged children (<6 years), school-aged children (6-15 years), and adolescents/adults (≥ 16 years). Overall, the age of the pre-school-aged children ranged from 3 months to 5.5 years with a mean of 3.2 years. There were slightly more girls than boys, but the difference was not statistically significant (130 versus 114; $p > 0.05$). Study participants from both villages showed similar age and sex profiles. For instance, the mean age of the three study groups in Azaguié Makouguié was 2.9 years (pre-school-aged children), 9.1 years (school-aged children), and 38.1 years (adolescents/adults), while the respective mean age in Azaguié M'Bromé were 3.3, 9.4 and 38.2 years. Whatever the age group considered, no statistical difference was found between sexes in both villages ($p > 0.05$).

In Azaguié Makouguié almost three-quarter of the mothers/guardians of pre-school-aged children were engaged in subsistence farming. Trading local goods was the second main occupation, as reported by 11.3% of the interviewees. In Azaguié M'Bromé, among the 122 mothers/guardians interviewed 59 (48.4%) and 38 (31.1%) were engaged in subsistence farming and local trading, respectively (Table 5.2).

Table 5.1: Characteristics of the study population in the two villages of Azaguié Makouguié and Azaguié M'Bromé, district of Azaguié, south of Côte d'Ivoire.

	Azaguié Makouguié					Azaguié M'Bromé					
		Male	Female				Male	Female			
Age group	n	No. (%)	No. (%)	<i>P</i> *	Mean age (SD)	n	No. (%)	No. (%)	<i>P</i> *	Mean age (SD)	<i>P</i> **
Total	242	125 (48.3)	117 (48.3)	0.675	17.6 (18.7)	259	116 (44.8)	143 (55.2)	0.170	15.1 (17.7)	0.881
<6 years	110	52 (47.3)	58 (52.7)	0.640	2.9 (1.3)	134	72 (53.7)	62 (46.3)	0.480	3.3 (1.5)	0.975
6-15 years	38	27 (71.1)	11 (28.9)	0.032	9.1 (2.7)	46	20 (43.5)	26 (56.5)	0.470	9.4 (2.7)	0.968
>15 years	94	46 (48.9)	48 (51.1)	0.866	38.1 (13.9)	79	34 (43.0)	45 (57.0)	0.312	38.2 (15.0)	0.990

* Pearson's χ^2 test, p value comparing males and females

** Kruskal-Wallis test, p value of mean age between villages

<6 years, pre-school-aged children; 6-15 years, school-aged children; >15 years, adolescents and adults

Table 5.2: Mothers' main activity, children care and water use practice.

	Azaguié	Azaguié	χ^2	P*
	Makouguié	M'Bromé		
	n (%)	n (%)		
Mother involved in subsistence farming	56 (70.0)	59 (48.4)	2.47	0.116
Mother involved in local trade	22 (27.5)	38 (31.1)	0.17	0.682
Mother with main activity strongly linked to water	22 (27.5)	24 (19.7)	1.05	0.306
Location of pre-school-aged children during main activity				
At the back of their mother	8 (36.4)	7 (29.2)	0.14	0.711
In the water	0	1 (4.2)	0.90	0.343
In close proximity to the water	1 (4.5)	4 (16.7)	1.41	0.235
At home with their older siblings	13 (59.1)	12 (50.0)	0.11	0.737
Knows about schistosomiasis	19 (23.8)	45 (37.2)	2.05	0.153
Knows about the place where one becomes infected	17 (89.5)	39 (88.6)	1.60	0.206
Commonly used water source for child toilet				
Traditional well	78 (97.5)	102 (85.0)	0.55	0.460

* Pearson's χ^2 test

Complete questionnaire data were available from 122 mothers in Azaguié M'Bromé and 80 in Azaguié Makouguié

5.4.3. Infection with *S. mansoni*

Figure 5.2 shows the prevalence and intensity of *S. mansoni* infection, stratified by age group and study village. According to quadruplicate Kato-Katz thick smears, the overall prevalence of *S. mansoni* was 42.7% with a prevalence of 46.3% in Azaguié Makouguié and 39.4% in Azaguié M'Bromé with no statistical difference between villages ($p = 0.324$).

In pre-school-aged children, based on a single Kato-Katz thick smear, the prevalence of *S. mansoni* was 14.3% ($n = 35$). Microscopic examination of quadruplicate Kato-Katz thick smears found 57 pre-school-aged children with *S. mansoni* eggs in their stool, 28 (25.5%) in Azaguié Makouguié and 29 (21.6%) in Azaguié M'Bromé. More than two-fold higher prevalences were found in school-aged children (Azaguié Makouguié: 68.4%, Azaguié M'Bromé: 58.7%) and adolescents/adults (61.7% and 59.5%, respectively). Three-quarter of the infected pre-school-aged children had light infections (1-99 EPG), whereas only six preschool-aged children had heavy *S. mansoni* infection (≥ 400 EPG), five in Azaguié M'Bromé and one in Azaguié Makouguié. None of the children below the age of 24 months were found *S. mansoni*-positive in Azaguié Makouguié, but two such individuals (6.9%) were found in Azaguié M'Bromé.

With regard to duplicate urine CCA cassette tests, including 'trace' as positive results, 89 (81.7%) of the pre-school-aged children were found infected with *S. mansoni* in Azaguié Makouguié and 96 (72.2%) in Azaguié M'Bromé. Considering 'trace' results as negative, the respective prevalences were 44.0% and 45.9%.

Table 5.3 summarizes the groups' arithmetic mean of *S. mansoni* (and *S. haematobium*) egg counts. No difference was found in the arithmetic mean fecal egg counts of *S. mansoni* between males and females and between villages, regardless of whether analysis was done for pre-school-aged children or all age classes combined (all $p > 0.05$). However, village-specific analysis revealed that female preschoolers and adolescents/adults in Azaguié Makouguié had statistically significantly higher *S. mansoni* fecal egg counts ($p < 0.001$), whereas in Azaguié M'Bromé, the fecal egg count of males in both the school-aged ($p < 0.001$) and the adolescents/adults groups were significantly higher compared to their female counterparts ($p = 0.008$).

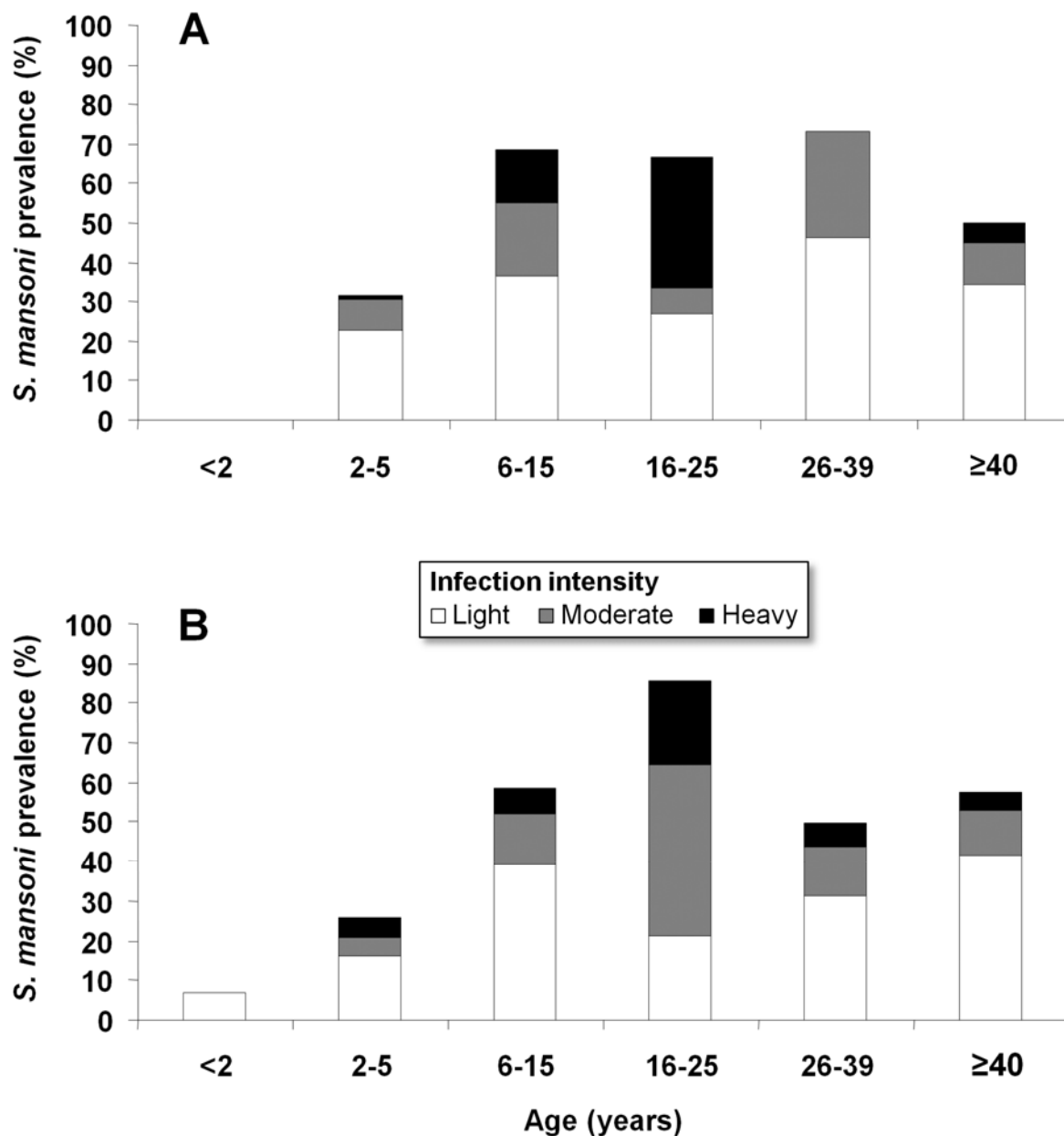


Figure 5.2: Prevalence and intensity categories of *S. mansoni* infection, stratified by age categories for Azaguié Makouguié (A) and Azaguié M'Bromé (B) in Azaguié district, south Côte d'Ivoire in mid-2011. *S. mansoni* infection intensities were categorized as light (1-99 EPG), moderate (100-399 EPG), and heavy (≥ 400 EPG).

5.4.4. Infection with *S. haematobium*

Figure 5.3 shows the prevalence and intensity of *S. haematobium* infection, stratified by age group and study village. In total, 62 individuals were found with *S. haematobium* eggs in their urine; hence, an overall prevalence of 12.4%. The prevalence of *S. haematobium* was considerably higher in Azaguié Makouguié compared to Azaguié M'Bromé (19.0% versus 6.2%). Among 110 pre-school-aged children in Azaguié Makouguié and 134 in Azaguié M'Bromé, based on duplicate urine filtrations, 19 (17.3%) and eight (5.9%) individuals were found with patent *S. haematobium* infections, respectively. The prevalence of *S. haematobium* was similar for males and females in both study villages ($p > 0.05$).

Visual inspection of urine among preschoolers revealed one case of macrohematuria, a child aged 2 years in Azaguié Makouguié. Nineteen (17.3%) and 14 (10.5%) pre-school-aged children had microhematuria in Azaguié Makouguié and Azaguié M'Bromé, respectively.

Among participants aged ≥ 6 years, seven school-aged children (18.4%) and 20 adolescents/adults (21.3%) were found with patent *S. haematobium* infection in Azaguié Makouguié. In Azaguié M'Bromé, five school-aged children (10.9%) and three adolescents/adults (3.8%) were infected with *S. haematobium*. In both villages, most of the individuals had light *S. haematobium* infections (1-49 eggs/10 ml of urine). No statistical difference was found between sex and between villages in arithmetic mean *S. haematobium* egg counts ($p > 0.05$).

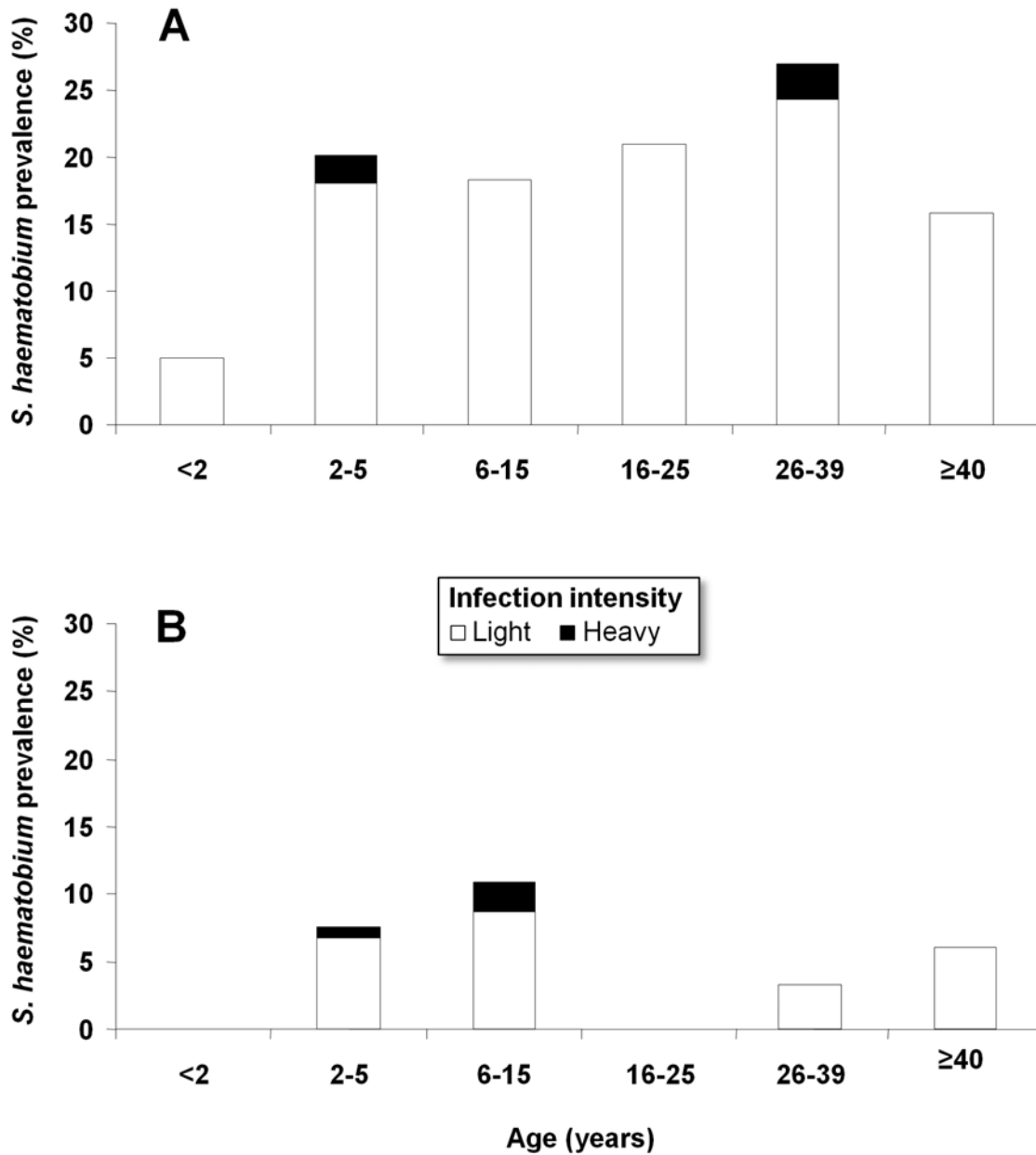


Figure 5.3: Prevalence and intensity categories of *S. haematobium* infection, stratified by age categories for Azaguié Makouguié (A) and Azaguié M'Bromé (B) in Azaguié district, south Côte d'Ivoire in mid-2011. *S. haematobium* infection intensities were categorized as light (1-49 eggs/10 ml of urine) and heavy (≥ 50 eggs/10 ml of urine).

Table 5.3: Arithmetic mean of *S. mansoni* and *S. haematobium* egg counts in pre-school-aged children (<6 years), school-aged children (6-15 years) and adolescents/adults (>15 years), stratified by sex and village.

	Azaguié Makouguié				Azaguié M'Bromé			
	Arithmetic mean				Arithmetic mean			
	n	Male	Female	<i>P</i> *	n	Male	Female	<i>P</i> *
<i>S. mansoni</i> (EPG)								
Total	242	69.1 (46.2-92.1)			259	59.4 (42.5-76.4)		0.561
<6 years	110	19.6 (0.5-38.6)	27.9 (6.4-49.4)	0.430	134	24.9 (1.8-48.0)	24.1 (3.9-44.2)	0.945
6-15 years	38	118.0 (1.5-234.7)	235.0 (0.0-482.4)	<0.001	46	112.3 (22.6-201.9)	64.7 (13.3-116.1)	<0.001
>15 years	94	55.3 (17.9-92.6)	120.4 (57.2-183.5)	<0.001	79	117.5 (48.3-186.6)	93.1 (44.7-141.5)	0.008
<i>S. haematobium</i> (eggs/10 ml of urine)								
Total	242	3.4 (1.9-4.9)			259	1.4 (0.8-2.1)		0.904
<6 years	110	2.4 (1.3-3.7)	4.9 (0.3-9.5)	0.812	134	1.1 (0.9-1.2)	1.3 (0.9-1.6)	0.986
6-15 years	38	3.2 (0.6-5.8)	5.6 (0.0-14.7)	0.699	46	5.4 (0.0-13.6)	1.0 (0.9-1.1)	0.52
>15 years	94	3.7 (0.0-8.4)	2..2 (1.4-3.0)	0.877	79	1.0 (0.9-1.04)	1.1 (0.9-1.1)	0.991

*Kruskal-Wallis test

5.4.5. *Other helminths and co-infection*

The overall prevalence of hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* in the two study villages was 14.2%, 5.4% and 2.4%, respectively. For each soil-transmitted helminth lower prevalences were found in Azaguié Makouguié compared to Azaguié M'Bromé (*T. trichiura*: 0.4% versus 10.0%; *A. lumbricoides*: 1.6% versus 3.1%, and hookworm: 11.6% versus 16.6%). Multiple species parasitic infections were common in both villages; 38.7% of the participants were infected with at least two helminth species concurrently. We found one 5-year-old boy in Azaguié M'Bromé who harboured a quadruplicate helminth species infection (i.e., *S. mansoni*, *S. haematobium*, *T. trichiura* and hookworm).

5.4.6. *Parasite association in pre-school-aged children*

Table 5.4 shows pairwise associations between *Schistosoma* infection, age, village, and mothers/guardians' behavioral factors. Infection with *S. mansoni* showed significant positive associations with *S. haematobium* (OR = 9.4, $p < 0.001$). Preschoolers with an age above 24 months were at a seven-fold higher odds to be infected with *S. mansoni* than their younger counterparts (<24 months). Pre-school-aged children staying at home when their mothers/guardians were involved in livelihood activities were at a two-fold higher odds of *S. mansoni* infection compared to those who accompanied their mothers/guardians. Infection with *S. haematobium* showed a significant positive association with *S. mansoni* (OR = 11.7, $p < 0.001$) and village (OR = 3.6, $p = 0.008$).

Table 5.4: Association between schistosome infections, adjusted by age, village, and mothers' behavioral factors.

Parasite	Association	Adjusted (95% CI)	OR <i>P</i>
<i>S. mansoni</i>			
	<i>S. haematobium</i>	9.4 (5.1-17.2)	<0.001
	Children aged <24 months (reference)	1.0	
	Age group (2-5 years)	8.8 (3.3-23.5)	<0.001
	Children accompanying their mothers to livelihood activities (reference)	1.0	
	Children stayed at home with their elders	2.3 (1.5-3.5)	0.017
<i>S. haematobium</i>			
	<i>S. mansoni</i>	11.7 (6.4-21.4)	<0.001
	M'Bromé (reference)	1.0	
	Makouguié	3.6 (1.9-6.6)	0.008

n = 244

Logistic regression was used to assess the association between schistosome (*S. mansoni* and *S. haematobium*) infections, adjusted by age and village. Association between *S. mansoni* or *S. haematobium* as outcome and *S. mansoni* or *S. haematobium*, age (< 24 months and 2-5 years) and village (Azaguié Makouguié and Azaguié M'Bromé) as explanatory variable. Age category "<24 months" was used as baseline for comparison of 2-5 years category. Azaguié M'Bromé was used as baseline for comparison of Azaguié Makouguié. *S. mansoni* and *S. haematobium* infections results were expressed as binary variable (positive/negative)

5.4.7. *Schistosomiasis risk maps*

Figure 5.4 Figure 4 shows the spatial distribution of *Schistosoma*-infected pre-school-aged children, as diagnosed by Kato-Katz (for *S. mansoni*) and urine filtration (for *S. haematobium*) and water contact points in Azaguié Makouguié and Azaguié M'Bromé. *Schistosoma*-infected pre-school-aged children were homogeneously distributed in Azaguié Makouguié. In Azaguié M'Bromé *Schistosoma* cases were clustered among pre-schoolers living in households in close proximity to water contact sites at higher risk of infection.

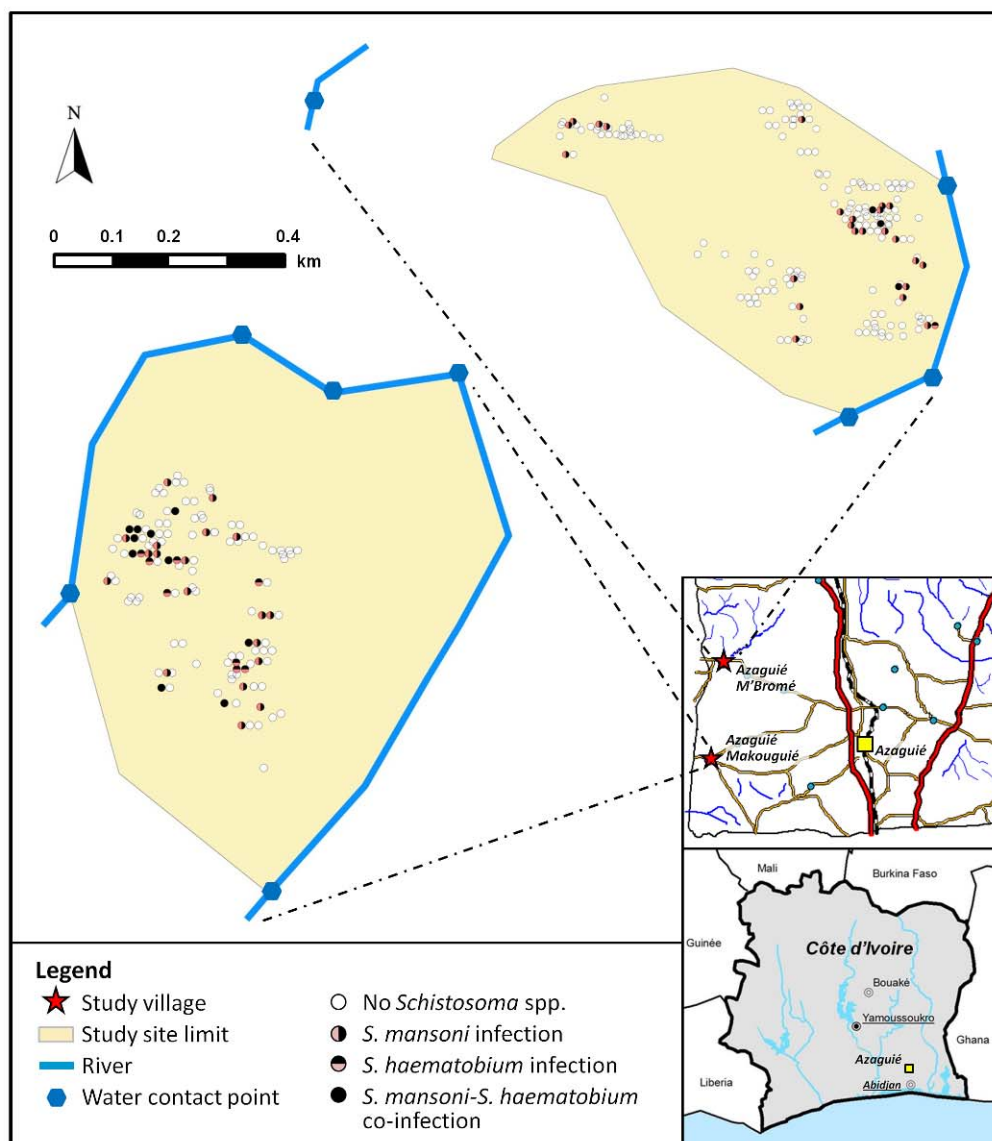


Figure 5.4: Map showing the town of Azaguié and its surrounding villages and settlements in south Côte d'Ivoire. The two villages where the current study was carried out are highlighted with an asterisk. The accompanying risk maps show water contact points and the spatial distribution of households inhabited by pre-school-aged children with no schistosome infection, mono-infection with either *S. mansoni* or *S. haematobium* or co-infection with both schistosome species, as determined by the Kato-Katz technique (for *S. mansoni*) and urine filtration (for *S. haematobium*).

5.4.8. *Results from the questionnaire and FGDs*

Table 5.2 summarizes the results obtained from the questionnaire administered to 202 mothers/guardians of pre-school-aged children in the two study villages. In Azaguié Makouguié 22 of the 80 mothers interviewed (27.5%) responded that their daily activities are strongly linked to water-contact patterns, somewhat higher than in Azaguié M'Bromé (24/122, 19.7%). In the latter village, half of these mothers reported that they take their preschoolers with them to the water contact sites. In Azaguié Makouguié the majority of preschoolers are left at home while mothers/guardians pursue their daily water chores (59.1%). Knowledge about schistosomiasis was poor; only 19 (23.8%) mothers in Azaguié Makouguié and 45 (37.3%) in Azaguié M'Bromé knew about the disease. On the other hand, most women knew that exposing children to water can bring about health risks (97.5% in Azaguié Makouguié and 85.0% in Azaguié M'Bromé). In both villages, the main source of drinking water comes from a traditional well.

FGDs revealed no difference in the patterns of water use between the mothers/guardians of *Schistosoma*-infected pre-school-aged children and their non-infected counterparts. Results from the FGDs highlighted that mothers give special care to children below the age of 24 months. FGDs also revealed that some individuals use local remedies to manage schistosomiasis, especially urogenital schistosomiasis. These local treatments were based on a mixture of plants and clay. The FGDs confirmed that most of the mothers lack detailed knowledge about schistosomiasis, but they know that contaminated water exposes their children to health risk.

5.5. Discussion

Studies going back as far as the 1960s documented that in specific social-ecological settings, young children before reaching school-age suffer from schistosomiasis (Smith, 1985; Farooq and Mallah, 1966; Siegal, 1968; Abdel-Salam and Abdel-Fattah, 1977; Perel et al., 1985). However, in view of peak infection prevalence and intensities usually observed in the school-aged population (Woolhouse, 1998), and the ease of implementing preventive chemotherapy through the education system, schistosomiasis control is centered on school-aged children. The lack of pharmacological data and an appropriate formulation of praziquantel for preschoolers are important reasons why this age group is largely excluded from preventive chemotherapy (Stothard et al., 2011a; Keiser et al., 2011). Yet, pre-school-aged children

might be particularly vulnerable to the negative consequences of an early-life infection with *Schistosoma* as they would not get treatment until entering school (Stothard and Gabrielli, 2007b). Additionally, adolescents and adults are given far less attention than the school-aged population when it comes to schistosomiasis control. Indeed, World Health Assembly (WHA) resolution 54.19 sets clear treatment coverage targets for the school-aged population, while it remains comparatively silent on other age groups.

Recent studies confirmed that pre-school-aged children are at risk of schistosomiasis, but in-depth epidemiologic investigations are few (Bosompem et al., 2004; Sousa-Figueiredo et al., 2008; Garba et al., 2010; Dabo et al., 2011; Ekpo et al., 2012). It has also been discussed whether preventive chemotherapy should be extended from school-aged to pre-school-aged children (Johansen et al., 2007; Stothard and Gabrielli, 2007a; Sousa-Figueiredo et al., 2010). However, there are a number of issues that must be addressed before policy recommendations can be made regarding the inclusion of preschoolers in preventive chemotherapy. First, what is the true extent of *Schistosoma* infection in pre-school-aged children in different epidemiologic settings? Second, what are the key risk factors that drive the epidemiology of schistosomiasis in pre-school-aged children? Third, can the prevalence and intensity of schistosomiasis in school-aged children serve as proxies for pre-school-aged children and adolescents/adults to better target control and enhance disease burden assessment?

The current study, pursuing a cross-sectional design, deepened our understanding of the epidemiology of schistosomiasis in two villages of south Côte d'Ivoire that are highly endemic for schistosomiasis, but have not been subjected to large-scale administration of praziquantel before. We employed a reasonably sensitive diagnostic approach with quadruplicate Kato-Katz thick smears for the diagnosis of *S. mansoni* and duplicate urine filtration for detection of *S. haematobium* eggs in all study participants. Additionally, in pre-school-aged children, a reagent strip was utilized for assessment of microhematuria, and a urine CCA cassette test was employed for diagnosis of *S. mansoni*.

Our study confirmed that pre-school-aged children are at risk of schistosomiasis. One boy, before reaching his first birthday (8 months), had a patent *S. mansoni* infection, as revealed by the Kato-Katz technique. Considering urine CCA test results, the earliest infection with *S. mansoni* was found in a boy aged only 3 months (Coulibaly et al., 2012). Previous studies have highlighted such early *Schistosoma* infection in areas of high endemicity (Sousa-Figueiredo et al., 2008; Ekpo et al., 2010; Namwanje et al., 2011; Stothard

et al., 2011a). The negative health impact of such early infections has been emphasized (Hotez et al., 2010; Sousa-Figueiredo et al., 2010; Stothard et al., 2011b). Our observation of CCA detected in the urine of a child as young as 3 months in the absence of *S. mansoni* eggs in fecal samples is in line with recent observations from a study in Uganda using different approaches for detecting *Schistosoma* infections in pre-school-aged children (Stothard et al., 2011b). It might be explained by CCA from the mother's colostrums (Odogwu et al., 2006) or from CCA produced by the young developing stages of the worm prior to patency, hence before egg production commences (Odogwu et al., 2006; Coulibaly et al., 2011) New research is needed to further elucidate this issue, which will be important to clarify operational research and control issues and help interpret diagnostic results. First, assuming that infants receive CCA from their mother's colostrums would jeopardize the use of urine CCA as a diagnostic assay in very young children. Indeed, based on positive urine CCA test results in the pre-school-aged population, one might suggest extending preventive chemotherapy to this age group. Second, if CCA are accrued from juvenile worms, this might be an early indicator allowing for efficient surveillance of schistosomiasis in endemic areas. However, since praziquantel is not effective against the young developing stages of *S. mansoni* (Sabah et al., 1986), treatment must be scheduled accordingly.

Overall, between 5.9% and 21.6% of the pre-school-aged children investigated had patent *S. haematobium* while the prevalence could be underestimated due to our urine sample collection approach and between 17.3% and 25.5% patent *S. mansoni* infections. Conversely, between 5.2% and 10.9% of the pre-school-aged children were co-infected, considerably higher than what would have been expected by chance (i.e., 1.0-5.5%). Our findings corroborate with recent results presented by Garba et al. (2010) from highly endemic villages in Niger (Garba et al., 2010). The consequences of dual species infections in the same body of pre-school-aged children have yet to be investigated.

In the present study most of the *S. mansoni* and *S. haematobium* infections in pre-school-aged children were of light intensity, which is in line with studies carried out elsewhere in sub-Saharan Africa (Odogwu et al., 2006; Stothard et al., 2011b). Nevertheless, we observed 12 (4.9%) pre-school-aged children heavily infected with either *S. mansoni* (≥ 400 EPG) or *S. haematobium* (≥ 50 eggs/10 ml of urine). All of these children were aged above 3 years. To our knowledge, pre-school-aged children never received praziquantel treatment in the current study settings. Hence, the heavy infections observed in 12 pre-

school-aged children above the age of 3 years is the likely result of very early infection, followed by cumulative infections over time (Stothard et al., 2011b).

For both *Schistosoma* species, we observed specific age-prevalence curves and intensities in both study villages. While it is commonly believed that the peak of *Schistosoma* infection prevalence occurs in children aged between 6 and 15 years (school-aged), age patterns in the intensity of infection are less clear-cut, with variations generally attributed to the level of contact with contaminated fresh water bodies (King, 2010; Verani et al., 2011). In our study, considering the burden of schistosomiasis as expressed by arithmetic mean egg counts, we concur that in both communities, pre-school-aged children, regardless of their sex, were similarly exposed to contaminated fresh water bodies. However, in school-aged children, adolescents, and adults, *S. mansoni* infection intensities were sex-dependent (higher risk in males), whereas for *S. haematobium* no sex difference was observed. Hence, socio-cultural factors might govern gender-specific water use (King, 2010). Additionally, setting-specific abiotic and biotic features govern the development of intermediate host snails. Taken together, the interplay of these factors could explain the observed age-prevalence and intensities profiles (Kariuki et al., 2004; Clennon et al., 2007).

In addition to our parasitological investigations, we also determined risk factors for schistosomiasis in pre-school-aged children, by conducting a series of FGDs with mothers and administration of a questionnaire. Interestingly, we found no difference in common water use practices between mothers of *Schistosoma*-infected and non-infected children. Hence, no specific behavior of mothers could explain the difference in the infection status of pre-school-aged children. The questionnaire showed that in both villages, most mothers left their children at home with their elder siblings when pursuing daily livelihood activities in the fields or on the market. Multivariable logistic regression revealed that pre-school-aged children staying at home were at a two-fold higher odd of *Schistosoma* infection than their counterparts accompanying their mothers during daily livelihood activities. It is conceivable that most of the infections in pre-school-aged children occur at times during which pre-school-aged children are taken along to the river by their elder siblings for swimming, bathing, washing, or fishing activities. FGDs revealed that mothers are aware that the river is an important source of ill-health, but the river remains essential for their many needs due to a lack of alternative water sources (for washing and drinking purposes). In the absence of a functioning health system, villagers use herbal remedies to treat schistosomiasis, particularly urogenital schistosomiasis.

In Azaguié Makouguié, we observed a rather homogenous distribution of *Schistosoma*-infected pre-school-aged children, whereas in Azaguié M'Bromé, there was clear clustering of schistosome infections in households located in close proximity to the main river. It is important to note that in Azaguié Makouguié only one traditional well exist. However, in Azaguié M'Bromé several water sources are available (several traditional wells and one water pump). It follows that a minimum improvement in safe water supply could significantly influence the risk for pre-school-aged children and other community members to be exposed to *Schistosoma* infections. Tchuem Tchuente and colleagues in 2001 (Tchuem Tchuente et al., 2001) showed that the installation of a single water pump in Kinding Ndjabi village, Cameroon, was an important feature in reducing schistosomiasis transmission.

5.6. Conclusion

Concluding, our study shows that schistosomiasis is governed by social-ecological contexts. Indeed, this parasitic disease is intimately linked with poverty, lack of essential social services and infrastructure (e.g., clean water, improved sanitation, hygiene, and health systems), and close proximity to small rivers and standing fresh water bodies upon which people depend for their daily occupational and recreational activities. Community members are at high risk of schistosomiasis and infections occur very early in childhood. Yet, in our study, prevalence and intensity of infection in the pre-school-aged children were relatively low, and hence, young children might warrant treatment just once before their 5th birthday. Improvement of water supply, sanitation, and hygiene, and strengthening of the health system are necessary to reduce people's exposure to infested fresh water bodies.

5.7. Acknowledgment

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6. Efficacy and safety of praziquantel in preschool-aged children in an area co-endemic for *Schistosoma mansoni* and *S. haematobium*

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6.1. Abstract

Background: In sub-Saharan Africa the recommended strategy to control schistosomiasis is preventive chemotherapy. Emphasis is placed on school-aged children, but in high endemicity areas, preschool-aged children are also at risk, and hence might need treatment with praziquantel. Since a pediatric formulation (e.g., syrup) is not available outside of Egypt, crushed praziquantel tablets are used, but the efficacy and safety of this treatment regimen is insufficiently studied.

Methodology: We assessed the efficacy and safety of crushed praziquantel tablets among preschool-aged children (<6 years) in the Azaguié district, south Côte d'Ivoire, where *Schistosoma mansoni* and *S. haematobium* coexist. Using a cross-sectional design, children provided two stool and two urine samples before and 3 weeks after treatment. Crushed praziquantel tablets, mixed with water, were administered at a dose of 40 mg/kg. Adverse events were assessed and graded 4 and 24 hours posttreatment by interviewing mothers/guardians.

Principal Findings: Overall, 160 preschool-aged children had at least one stool and one urine sample examined with duplicate Kato-Katz thick smears and a point-of-care circulating cathodic antigen (POC-CCA) cassette for *S. mansoni*, and urine filtration for *S. haematobium* diagnosis before and 3 weeks after praziquantel administration. According to the Kato-Katz and urine filtration results, we found high efficacy against *S. mansoni* (cure rate (CR), 88.6%; egg reduction rate (ERR), 96.7%) and *S. haematobium* (CR, 88.9%; ERR, 98.0%). POC-CCA revealed considerably lower efficacy against *S. mansoni* (CR, 53.8%). Treatment was generally well tolerated, but moderately severe adverse events (i.e., body and face inflammation), were observed in four *Schistosoma* egg-negative children.

Conclusions/Significance: Crushed praziquantel administered to preschool-aged children at a dose of 40 mg/kg is efficacious against *S. mansoni* and *S. haematobium* in a co-endemic setting of Côte d'Ivoire. Further research is required with highly sensitive diagnostic tools and safety must be investigated in more depth.

Clinical Trial Registration: Current Controlled Trial (identifier: *ISRCTN 53172722*).

6.2. Author summary

Schistosomiasis is a parasitic worm infection that plagues more than 200 million people in the developing world, particularly in sub-Saharan Africa. The current strategy to control schistosomiasis is to regularly administer the deworming drug praziquantel to school-aged children. Younger children before reaching school-age are not included in these deworming campaigns, because they are considered at low risk of schistosomiasis, and because the amount of available data to evaluate the safety of praziquantel in young children is insufficient. We conducted a study in two villages in southern Côte d'Ivoire and examined the stool and urine of more than 250 children (<6 years) for schistosome eggs and antigens. Children were treated with crushed praziquantel tablets (40 mg/kg) and the efficacy of this treatment was determined 3 weeks after treatment. The safety of the treatment was assessed by interviewing mothers of treated children for adverse events (e.g., abdominal pain, diarrhea, and headache). Complete data records were available for 160 children. Praziquantel cleared most of the infections. The treatment was generally well tolerated, but we observed four children who were not infected at the baseline survey who developed face and body inflammation that required close supervision by the study physician.

6.3. Introduction

Schistosomiasis is still a major public health problem in many parts of the developing world, especially in sub-Saharan Africa (King et al., 2005; Gryseels et al., 2006; Steinmann et al., 2006; Utzinger et al., 2009; Gray et al., 2011). Indeed, more than 200 million people are infected, with about half of them suffering from morbid sequelae, including hematuria, dysuria, nutritional deficiencies, anemia, growth retardation, and decreased physical performance and cognitive development (Stephenson et al., 1985; Stoltzfus et al., 1997; Jukes et al., 2002; Bhargava et al., 2003; King et al., 2005). The anthelmintic drug praziquantel is the cornerstone for morbidity control with millions of people treated every year (Fenwick et al., 2003; WHO, 2002; Utzinger and Keiser, 2004; Doenhoff et al., 2008). Morbidity control is emphasized since the mid-1980s, and this strategy has been reinforced in 2001 by the World Health Assembly (WHA) resolution 54.19, which urged member states to regularly deworm at least 75% and up to 100% of school-aged children at risk of schistosomiasis and soil-transmitted helminthiasis (WHO, 2002; Savioli et al., 2009).

Preschool-aged children (individuals below the age of 5-6 years) are currently excluded from preventive chemotherapy control campaigns. The main reasons for this exclusion are that preschool-aged children are believed to be at low risk of schistosomiasis (Jordan et al., 1993) and that there is insufficient documentation on the safety of praziquantel in this age group (Stothard et al., 2011; Keiser et al., 2011). However, recent studies carried out in different parts of East and West Africa showed that in high endemicity areas a considerable proportion of preschool-aged children are infected with *Schistosoma*, and hence treatment might need to be extended to younger age groups in such high-risk areas (Mafiana et al., 2003; Bosompem et al., 2004; Odogwu et al., 2006; Sousa-Figueiredo et al., 2008; Garba et al., 2010; Sousa-Figueiredo et al., 2010a; Sousa-Figueiredo et al., 2010b; Dabo et al., 2011; Ekpo et al., 2012).

With regard to morbidity control of schistosomiasis using praziquantel, it is important to note that an appropriate pediatric formulation for treating preschool-aged children is currently not available outside of Egypt (i.e., praziquantel syrup, Epiquantel, manufactured by the Egyptian International Pharmaceutical Industries Co. A.R.E., Cairo, Egypt). Hence, a common approach in high endemicity areas is to use praziquantel tablets (600 mg), crush them between two spoons, mix with water or fruit juice, and then administer orally to preschool-aged children at a dose of 40 mg/kg (Stothard et al., 2011; Sousa-Figueiredo et al., 2010b). Recent studies using Epiquantel in preschool-aged children revealed similar efficacy than crushed praziquantel tablets (WHO, 2010; Navaratnam et al., 2012).

The study reported here was designed to assess the efficacy and safety of crushed praziquantel tablets in preschool-aged children (<6 years) in an area where *Schistosoma mansoni* and *S. haematobium* coexist (Coulibaly et al., 2011; Coulibaly et al., 2012). Our findings, along with other investigations pertaining to the epidemiology of schistosomiasis in preschool-aged children, may be useful to further optimize the control of schistosomiasis in a currently neglected age group.

6.4. Methods

6.4.1. Ethics statement

Ethical approval was granted by the Ministry of Health and Public Hygiene of Côte d'Ivoire (reference no. 4248/2010/MSHP/CNER). The trial is registered with ClinicalTrial.gov (identifier: ISRCTN 53172722) and the protocol is available as Supporting Information

(Protocol S1). Local authorities of the Azaguié district were informed about the purpose, procedures, and potential risks and benefits of the study. In the absence of recent census data, an exhaustive door-to-door survey was carried out to identify all preschool-aged children (<6 years) in the two selected villages. Parents or guardians of eligible children were informed about the objectives of the study and asked to provide written informed consent on behalf of their children. Only those preschool-aged children who had written informed consent from their parents/guardians were included.

Participation was voluntary and parents/guardians could withdraw their child from the study anytime without further obligation. At the end of the study, anthelmintic drugs (i.e., praziquantel against schistosomiasis and albendazole against soil-transmitted helminthiasis) were offered free of charge to all community members.

6.4.2. *Study area and population*

The study was conducted between June and November 2011 in two villages located in the district of Azaguié, southern Côte d'Ivoire: Azaguié Makouguié (geographical co-ordinates 05°37'33" N latitude, 04°09'04" W longitude) and Azaguié M'Bromé (05°39'42" N, 04°08'38" W). Recent studies have shown that *S. mansoni* and *S. haematobium* are coendemic in Azaguié (N'Guessan et al., 2007; Coulibaly et al., 2011). Villagers are mainly engaged in subsistence farming. Both villages lack access to clean water and improved sanitation.

6.4.3. *Study design and sample size*

We pursued an intervention study (i.e., praziquantel administration), including children of both sexes below the age of 6 years. Children's infection with *S. mansoni* and *S. haematobium* was assessed during a baseline cross-sectional survey and again 3 weeks posttreatment using standardized, quality-controlled methods. The STROBE checklist is available as Supporting Information (Checklist S1).

With the overarching goal to further the understanding of the epidemiology, diagnosis, and control of schistosomiasis in preschool-aged children, we aimed at a sample of about 200 individuals, as recommended by statistical textbooks in health studies (Hsieh and Liu, 1990). Our population census carried out in June 2011 revealed 367 preschool-age children. We assumed that the prevalence of *S. mansoni* in preschool-aged children would be around 20% (i.e., a quarter of the 80% *S. mansoni* infection prevalence observed in school-aged children in

Azaguié in 2010 (Coulibaly et al., 2011)) and that about 70% of the preschool-aged children would comply (lower rate than among school-aged children due to the difficulty to obtain biological samples in this younger age group). Aiming for a precision of 5%, we finally decided to include all 367 registered preschool-aged children.

6.4.4. *Inclusion criteria*

We adhered to the following inclusion criteria: (i) preschool-aged children (<6 years); (ii) written informed consent by parents/guardians; (iii) submission of at least one sufficiently large stool sample for duplicate Kato-Katz thick smears, and one urine sample for a 10 ml filtrate and a single point-of-care circulating cathodic antigen (POC-CCA) cassette test; (iv) no abnormal medical condition, as judged by the study physician on the day of the treatment; (v) no recent anthelmintic treatment (within the past 4 weeks) according to a parental questionnaire; and (vi) no participation in any other clinical trial.

6.4.5. *Parasitological and clinical examinations*

The door-to-door census carried out in June 2011 to establish up-to-date census data generated lists of preschool-aged children, including their name, age, sex, and geographical coordinates of the household. Mothers/guardians of the preschool-aged children were provided with plastic containers labeled with unique identifiers (IDs) and they were asked to obtain a fresh stool and urine sample of their child. Stool and urine samples were collected at any time of the day due to the difficulty of collecting biological samples in this age group. Our aim was to obtain two stool and two urine samples over two consecutive days from each participating child.

6.4.6. *Laboratory procedures*

Stool and urine samples were transferred to a nearby laboratory in Azaguié town and worked up on the day of collection. For the diagnosis of *S. mansoni* and soil-transmitted helminths, duplicate Kato-Katz thick smears were prepared from each stool sample (i.e., quadruplicate Kato-Katz thick smears per child), using 41.7 mg templates (Katz et al., 1972). The Kato-Katz thick smears were allowed to clear for at least 30 min before examination under a microscope by experienced laboratory technicians. The number of *S. mansoni* and soil-transmitted helminth eggs were counted and recorded for each species separately.

For the diagnosis of *S. haematobium*, urine samples were subjected to a filtration method, as described elsewhere (Utzing et al., 2011). Briefly, a single filtration was performed with each urine sample (i.e., two urine filtrations per child over two consecutive days). Urine samples were vigorously shaken and 10 ml pressed through a small-meshed filter (aperture: 30 µm) and a drop of Lugol's solution was added on the filter paper, which was then placed onto a microscope slide. Slides were examined under a microscope and the number of *S. haematobium* eggs counted by experienced technicians.

For quality control, 10% of the Kato-Katz thick smears and the urine filter slides were re-examined by a senior technician. In case of disagreement, the results were discussed with the concerned technician and discordant slides re-read until agreement was reached.

An additional approach for the diagnosis of *S. mansoni* was employed, namely a POC-CCA cassette, that is based on a commercially available lateral flow immuno-chromatographic test (Coulibaly et al., 2011). The POC-CCA cassette (batch 33112) was performed according to the manufacturer's instructions. In brief, a drop of urine was added to the well and once fully absorbed a drop of buffer was added. The tests were read within 20-25 min. In case the control band failed to develop, the test was considered invalid and the respective urine sample retested with a new POC-CCA. Valid tests were scored as negative or positive, the latter stratified into trace (very light color band), 1+, 2+, and 3+ according to the visibility of the color reaction. The tests were scored independently by two investigators. In case of conflicting results, a third investigator was consulted, and the results discussed until agreement was reached (Coulibaly et al., 2011).

6.4.7. Praziquantel treatment and monitoring of adverse events

Children were treated with crushed praziquantel tablets (600 mg; Biltricide, Bayer) at a dose of 40 mg/kg (Fenwick et al., 2003). Children were weighed using an electronic balance (Evolis; Rumily, France). The appropriate number of praziquantel tablets (e.g., half a tablet for a child weighing 7-8 kg) were crushed between two spoons, mixed with tap water in a clean soup spoon before oral administration. Treatment was given by the mothers/guardians of the children under close supervision of trained medical personnel. Children were closely monitored by medical staff for 4 hours. In case vomiting occurred within 1 hour after treatment, a second dose of praziquantel was administered.

Treatment-related adverse events were assessed 4 and 24 hours posttreatment. Mothers/guardians were asked to report unusual behavior of their children since drug intake,

and whether any of the following adverse events occurred: abdominal pain, allergic reaction, diarrhea, dizziness, fatigue, fever, headache, nausea, and vomiting. Adverse events were graded (i.e., light, moderate, severe, or life threatening), as described elsewhere (Keiser et al., 2010).

6.4.8. *Treatment efficacy evaluation*

Three weeks after praziquantel administration, stool and urine samples were collected again, using the same procedures. Treatment efficacy was determined by means of cure rate (CR, percentage of children positive at the pretreatment cross-sectional survey who became egg-negative 3 weeks after treatment, as assessed by the Kato-Katz technique for *S. mansoni* and urine filtration for *S. haematobium*) and egg reduction rate (ERR, reduction in the group's geometric mean fecal egg count for *S. mansoni* or the group's geometric mean *S. haematobium* egg count in 10 ml of urine comparing the before and after treatment situation).

6.4.9. *Statistical analysis*

Data were double entered into an Excel spreadsheet, transferred into EpiInfo version 3.2 (Centers for Disease Control and Prevention; Atlanta, USA) and cross-checked. Statistical analyses were done with Stata version 10 (Stata Corp.; College Station, USA). Preschool-aged children who had at least one stool sample examined with duplicate Kato-Katz thick smears, a single POC-CCA cassette for *S. mansoni* diagnosis, and one urine sample subjected to a filtration method for *S. haematobium* diagnosis before and after treatment were included in the final analysis (per-protocol). Continuous data (e.g., schistosome egg counts) are presented as geometric mean, whereas dichotomous data (e.g., presence or absence of an infection) are presented as proportion.

Infection intensities were stratified according to cut-offs proposed by the World Health Organization (WHO) (Fenwick et al., 2003). There are three intensity classes for *S. mansoni*: (i) light (i.e., 1-99 eggs/gram of stool (EPG)); (ii) moderate (100-399 EPG); and (iii) heavy (≥ 400 EPG). *S. haematobium* infections were categorized as light (1-49 eggs/10 ml of urine) and heavy (≥ 50 eggs/10 ml of urine).

6.5. Results

6.5.1. *Adherence and population characteristics*

Figure 6.1 shows the adherence of preschool-aged children to the study protocol. The village census revealed 367 children aged below 6 years, all of whom were invited to participate. Sixty-three children were absent during the baseline survey and 16 had no written informed consent by their parents/guardians. From the 288 children participating at the baseline cross-sectional survey, seven were excluded due to incomplete parasitological data (e.g., insufficiently large stool sample for duplicate Kato-Katz thick smears). Among the remaining 281 children, 234 were administered crushed praziquantel. Three weeks posttreatment, we were able to re-examine at least one sufficiently large stool and urine sample from 160 children.

Our final study cohort consisted of 82 (51.3%) boys and 78 girls with an average age of 3.2 years (range: 5 months to 5 years). Boys were slightly younger than girls (average, 3.0 years; 95% confidence interval (CI), 2.7-3.3 years versus average, 3.3 years; 95% CI, 3.0-3.6 years).

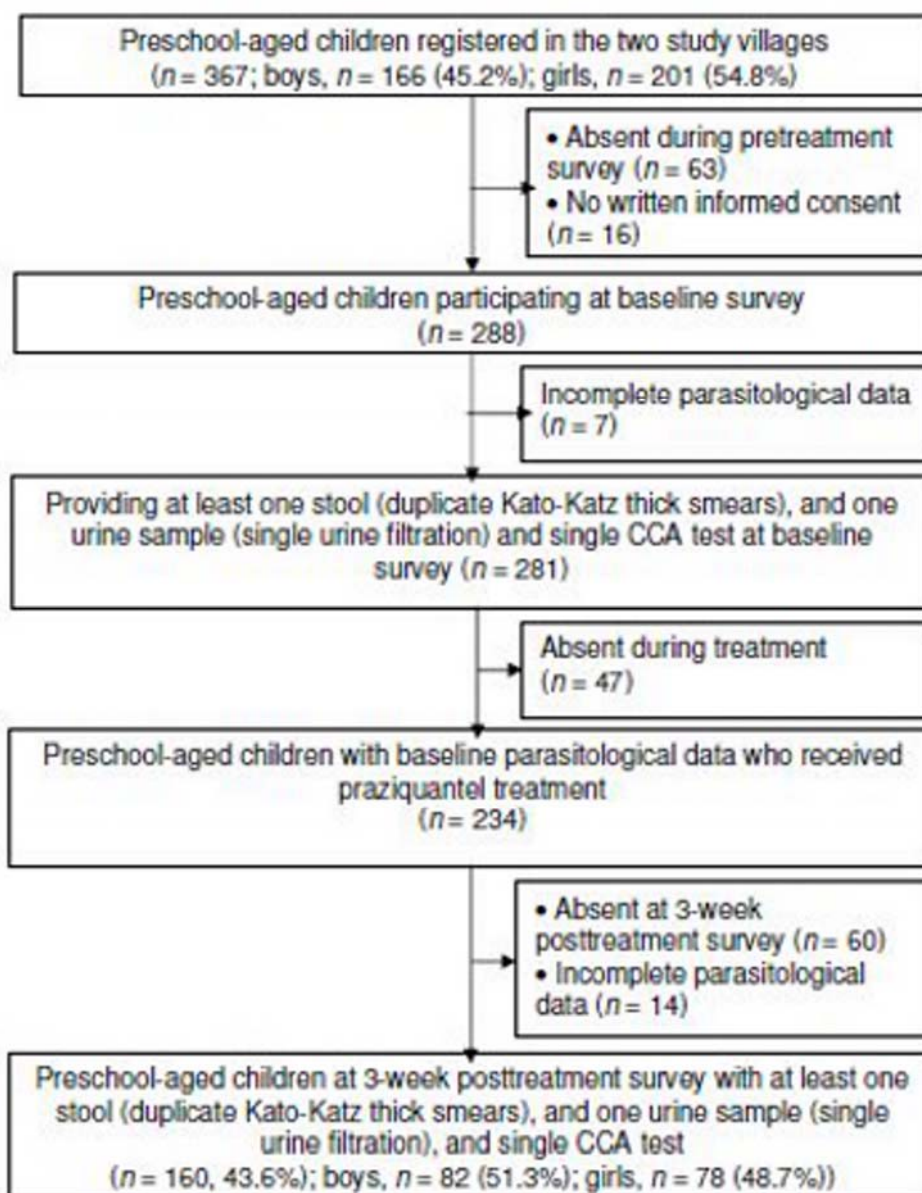


Figure 6.1: Flow chart and study adherence. The study was carried out in the villages of Azaguié Makouguié and Azaguié M'Bromé in south Côte d'Ivoire, between June and November 2011.

6.5.2. *Baseline characteristics*

Table 6.1 shows the pretreatment *S. mansoni* and *S. haematobium* infections, stratified by children's sex and diagnostic approach. According to at least duplicate Kato-Katz thick smears, 35 children of our per-protocol population (21.9%) were found *S. mansoni*-positive, with a geometric mean infection intensity of 1.2 EPG (Table 2). According to the POC-CCA results, there were 128 *S. mansoni* infections (80.0%) when considering 'trace' results as positive, and 78 (48.7%) considering 'trace' results as negative. With regard to *S. haematobium*, the urine filtration method revealed 18 infections (11.2%). The geometric mean infection intensity was 1.0 eggs/10 ml of urine. Eleven children (6.9%) were co-infected with *S. mansoni* and *S. haematobium*.

The Kato-Katz technique also allows detection of soil-transmitted helminth eggs. The observed prevalence of *Trichuris trichiura*, hookworm and *Ascaris lumbricoides* was 10.9%, 5.9% and 3.8%, respectively.

Table 6.1: Baseline prevalence of *S. mansoni* and *S. haematobium*, stratified by sex and diagnostic approach.

Species	Diagnostic approach	No. of children examined	No. (%) of infected children
<i>S. mansoni</i>	Kato-Katz		
	Male	82	19 (23.2)
	Female	78	16 (20.5)
	Total	160	35 (21.9)
	CCA test (incl. trace)		
	Male	82	68 (82.9)
	Female	78	60 (76.9)
	Total	160	128 (80.0)
	CCA test (excl. trace)		
	Male	82	44 (53.7)
	Female	78	34 (43.6)
	Total	160	78 (48.7)
<i>S. haematobium</i>	Urine filtration		
	Male	82	9 (10.9)
	Female	78	9 (11.5)
	Total	160	18 (11.2)

The study was carried out in two villages in Azaguié, south Côte d'Ivoire, between June and November 2011, focusing on preschool-aged children

6.5.3. Praziquantel efficacy

Table 6.2 summarizes CR and ERR, stratified by diagnostic approach. According to the Kato-Katz technique, at the 3-week posttreatment follow-up, four children (2.5%) were identified with *S. mansoni* eggs in their stool with a geometric mean infection intensity of 0.04 EPG. The CR and ERR was 88.6% and 96.7%, respectively. The POC-CCA results only allowed estimating CR. Including or excluding ‘traces’ as positive results, revealed 79 and 36 *S. mansoni*-infected children, respectively, at the 3-week posttreatment follow-up. The respective CRs were 38.3% and 53.8%.

With regard to *S. haematobium*, two children (1.3%) had a positive urine filtration at the 3-week posttreatment follow-up with a geometric mean infection intensity of 0.02 eggs/10 ml of urine. The CR and ERR were 88.9% and 98.0%, respectively.

Figure 6.2 shows infection intensity categories of *S. mansoni* and *S. haematobium* at the baseline and posttreatment surveys. At baseline, among 35 *S. mansoni*-infected children, 23, nine and three children had light, moderate and heavy infections, respectively. With regard to *S. haematobium*, 17 children and one child were lightly or heavily infected, respectively. At the 3-week posttreatment follow-up all children who were still *Schistosoma*-positive had light infections.

Table 6.2: Cure and egg reduction rates after praziquantel treatment, stratified by diagnostic approach.

Species	Diagnostic approach	Baseline	Follow-up	CR in % (95% CI)	Geometric mean		
		No. (%) of infected children	No. (%) of infected children		Baseline	Follow-up	ERR in %
<i>S. mansoni</i>	Kato-Katz	35 (21.9)	4 (2.5)	88.6 (73.3-96.8)	1.2 (0.9-1.4) EPG	0.04 (0-0.09) EPG	96.7
	CCA test (incl. trace)	128 (80.0)	79 (49.4)	38.3 (29.8-47.3)	NA	NA	NA
	CCA test (excl. trace)	78 (48.7)	36 (22.5)	53.8 (42.2-65.2)	NA	NA	NA
<i>S. haematobium</i>	Urine filtration	18 (11.2)	2 (1.3)	88.9 (65.3-98.6)	1.0 (0.9-1.1) eggs/10 ml urine	0.02 (0-0.06) eggs/10 ml urine	98.0

The study was carried out in two villages in Azaguié, south Côte d'Ivoire, between June and November 2011, focusing on preschool-aged children.

CCA, circulating cathodic antigen; CI, confidence interval; EPG, eggs per gram of stool; NA, not applicable

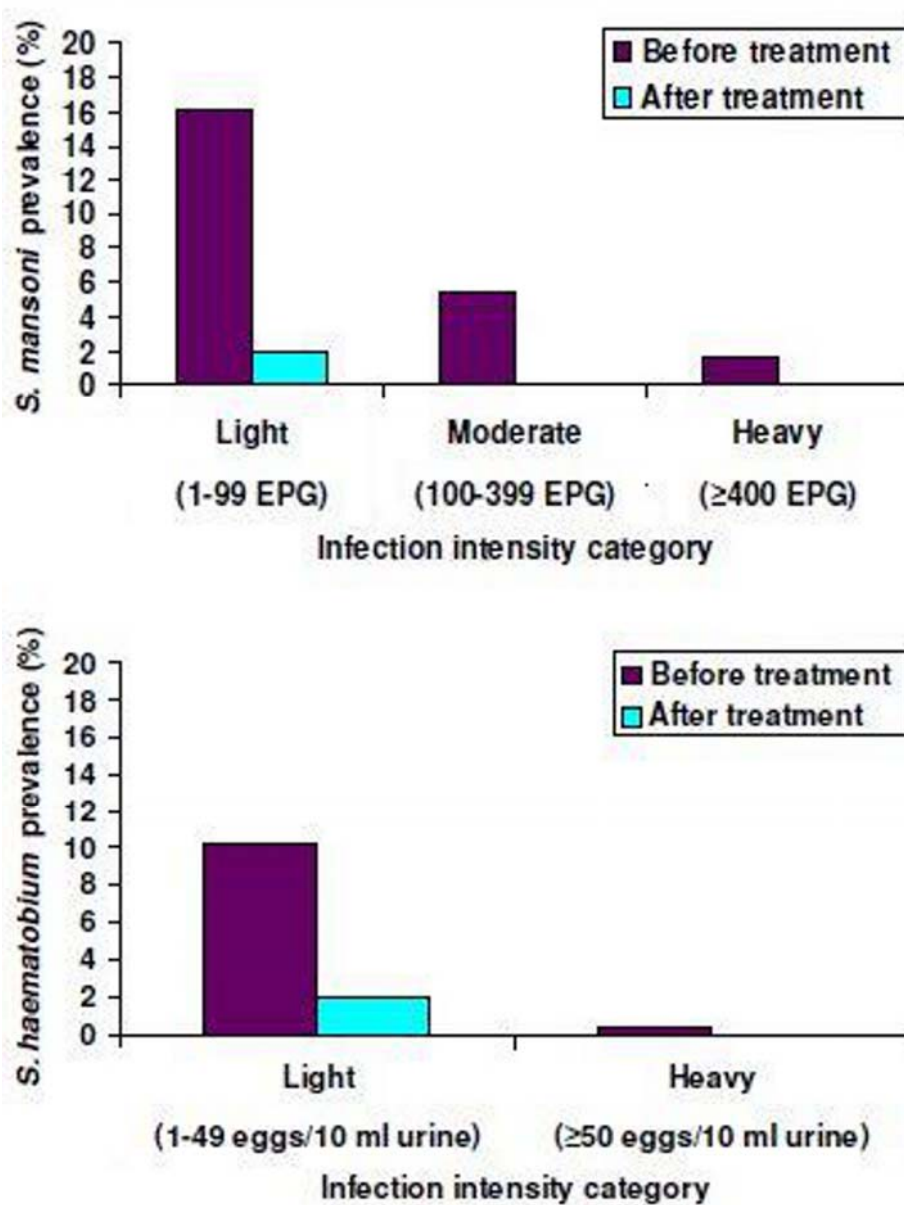


Figure 6.2: Infection intensity categories of *S. mansoni* and *S. haematobium* before and after praziquantel administration. Parasitological results are based on duplicate Kato-Katz thick smears examination (for *S. mansoni*) and urine filtration (for *S. haematobium*).

6.5.4. Adverse events

Table 6.3 shows the incidence of adverse events 4 and 24 hours after praziquantel administration among the 234 treated children. Overall, 43 children reported to have adverse events. Among these children, 12 (27.9%) were coinfecting with *S. mansoni* and *S. haematobium*, 17 (39.5%) had *S. mansoni* single infection, no child was infected with *S. haematobium* only, and 14 children (32.6%) were not infected at all. We stratified adverse events into two independent groups. The first group designated children with adverse events reported within 4 hours posttreatment and the second group was made up by children with adverse events reported by their mothers/guardians 24 hours posttreatment. Most of the adverse events were observed within the first 4 hours after treatment (n = 32, 74.4%), including abdominal pain (n = 7), diarrhea (n = 6), nausea (n = 5), vomiting (n = 4), dizziness (n = 3), fever (n = 2), fatigue (n = 2), face and body inflammation (n = 2), and headache (n = 1). More than one adverse event was observed in three (abdominal pain and diarrhea), two (vomiting and nausea) and one (fever and dizziness) children within 4 hours posttreatment. Twenty-four hours posttreatment, 11 (25.6 %) children reported adverse events, including diarrhea (n = 3), abdominal pain (n = 2), fatigue (n = 2), face and body inflammation (n = 2), dizziness (n = 1), and headache (n = 1). At this time point, none of the children reported multiple adverse events.

Adverse events were considered of light severity, with the only exception of face and body inflammation that was graded as a moderately severe. Mothers/guardians with children experiencing body and face inflammation sought advice from the study physician who assured the mothers that this adverse event is transient and self-limiting. Indeed, within 24 hours, children's conditions resolved to normal. The four children complaining of body and face inflammation were all *Schistosoma* egg-negative according to Kato-Katz and urine filtration results, but one had a positive POC-CCA test results (1+).

Table 6.3: Adverse events 4 and 24 hours after administration of crushed praziquantel tablets (n = 160).

Adverse event	Severity	Incidence (%)	
		4 hours	24 hours
Abdominal pain	Light	5 (3.1)	1 (0.6)
Vomiting		4 (2.5)	0
Diarrhea		3 (1.9)	3 (1.9)
Dizziness		3 (1.9)	0
Fatigue		2 (1.3)	2 (1.3)
Nausea		2 (1.3)	0
Fever		1 (0.6)	0
Headache		0	1 (0.6)
Body and face inflammation	Moderate	2 (1.3)	2 (1.3)
Total		22	9

The study was carried out in two villages in Azaguié, south Côte d'Ivoire between June and November 2011, focusing on preschool-aged children

6.6. Discussion

The anthelmintic drug praziquantel is the cornerstone for morbidity control due to schistosomiasis (WHO, 2002; Utzinger and Keiser, 2004; Doenhoff et al., 2008; Savioli et al., 2009). Emphasis is placed on school-aged children, whereas preschool-aged children (individuals below the age of 5-6 years) are usually excluded from preventive chemotherapy. However, in highly endemic areas, a considerable amount of preschool-aged children is already affected by schistosomiasis (Bosompem et al., 2004; Sousa-Figueiredo et al., 2008; Sousa-Figueiredo et al., 2010b; Ekpo et al., 2012). For that reason, there is ongoing discussion whether preventive chemotherapy with praziquantel should be extended to preschoolers (Sousa-Figueiredo et al., 2010a; Stothard et al., 2011; Navaratnam et al., 2012; Sousa-Figueiredo et al., 2012). However, absorption and metabolism of drugs are age-dependent (Keiser et al., 2011) and it is not well understood whether the developmental changes in the physiology during biological maturation from newborns to adolescence influence the efficacy and toxicity of praziquantel. We assessed the efficacy and safety of crushed praziquantel among preschool-aged children in an area of south Côte d'Ivoire where *S. mansoni* and *S. haematobium* coexist (Coulibaly et al., 2011).

Our study confirms that preschool-aged children are at risk of schistosomiasis. Indeed, we found that more than 20% of the children before their sixth birthday were infected with either *S. mansoni* or *S. haematobium*, or both species concurrently (7%). Using a commercially available POC-CCA cassette test, more than half of the children showed positive *S. mansoni* antigen reactions. Our study reveals that crushed praziquantel (40 mg/kg) administered to preschool-aged children is highly efficacious with CRs around 90% and ERRs above 95%, when standard diagnostic methods (Kato-Katz for *S. mansoni* and urine filtration for *S. haematobium*) were used.

There are two limitations of our study worth highlighting. First, we initially aimed to obtain two stool samples (for quadruplicate Kato-Katz thick smears) and two urine samples (for duplicate urine filtration and duplicate POC-CCA cassette test) from each participant before and after praziquantel administration. However, it proved difficult to obtain multiple stool and urine samples in this age group, and hence we finally included preschool-aged children who had at least duplicate Kato-Katz thick smears and a single urine filtration before and after treatment. In view of this sampling effort and diagnostic approach, it is clear that we have missed some infections, particularly those of light intensity. Second, a considerable proportion of preschool-aged children were lost to follow-up. However, comparing our per-

protocol population (n = 160) with children with incomplete parasitological data, who were absent at treatment, or lost to follow-up (n = 121), we found comparable prevalence estimates and infection intensities with *S. mansoni* and *S. haematobium*.

In spite of the aforementioned limitations, our investigation provides new insight into the efficacy and safety of praziquantel among a neglected population group in a *S. mansoni*-*S. haematobium* co-endemic area. Our findings support previous studies conducted in other parts in Africa. For example, a recent study carried out in preschool-aged children in Uganda, using quadruplicate Kato-Katz thick smears (two stool samples, each examined by duplicate Kato-Katz), reported a slightly lower efficacy against *S. mansoni* (CR, 80.2%; ERR, 87.9%) (Stothard et al., 2011). Mutapi and colleagues in a study done in Zimbabwe with children aged 1-5 years found high CR (92%) and very high ERR (99%) of crushed praziquantel against *S. haematobium* (Mutapi et al., 2011). Of considerable concern are recent findings from Ugandan preschool-aged children, as the overall CR among 305 *S. mansoni* egg-patent individuals was only 56.4%, with particularly low CR observed in preschoolers with a history of previous praziquantel treatments (CR 41.7%) (Sousa-Figueiredo et al., 2012). It should also be noted that the process of crushing praziquantel tablets is time consuming, and hence poses a challenge for large-scale control programs. It is encouraging to note that efforts are under way to develop a pediatric formulation of praziquantel, for example oro-dispersable tablets or minitables that might be more convenient to administer to small children (WHO, 2010).

In our study, we not only used standard diagnostic tests (i.e., Kato-Katz and urine filtration) but also a more recently developed and now commercially available POC-CCA cassette applied to urine for the diagnosis of *S. mansoni* (Rapid Medical Diagnostics, Pretoria, South Africa). Considering POC-CCA results from the pre- and posttreatment surveys, including or excluding ‘trace’ results as positive, we found low CRs of 38.0% and 53.8%, respectively. These results are worrying and reasons explaining the differences in CR according to the diagnostic technique might be explained as follows. First, *S. mansoni* eggs might have been missed by the Kato-Katz technique, particularly at the posttreatment follow-up when the remaining positive children had very low infection intensities. Indeed, it is widely acknowledged that the Kato-Katz technique lacks sensitivity in areas characterized by low *S. mansoni* infection intensities, which is common after treatment (Utzing et al., 2001). Second, perhaps CCA might still be detectable in urine 3 weeks after treatment (Nibbeling et al., 1998; Legesse and Erko, 2007), while the recommended time for *S. mansoni* assessment after treatment is 15-20 days (Scherrer et al., 2009). Third, while children might have been

cured from patent *S. mansoni* infection, praziquantel is largely refractory against young developing stages of the worms (Utzinger et al., 2004; Doenhoff et al., 2008), and hence antigens might still be present in the urines of young children. Fourth, the POC-CCA cassette might lack specificity after praziquantel administration. Further investigations are therefore needed to determine whether or not a POC-CCA cassette can be utilized for determining praziquantel efficacy, including the most appropriate time point posttreatment. Three weeks posttreatment might be too short for assessing praziquantel efficacy in preschool-aged children, but the longer one waits, the higher the risk for confounding factors (e.g., schistosomula fully developed into adult worms, and reinfection).

In our study, we also thoroughly assessed the safety of crushed praziquantel given to preschoolers. We observed similar frequencies of adverse events in preschool-aged children as reported by other groups (Dabo et al., 2011; Mutapi et al., 2011), and as observed in school-aged children (Raso et al., 2004). Recently, Namwanje and colleagues showed that praziquantel alone and in combination with mebendazole in the treatment of *S. mansoni* and soil-transmitted helminths in preschool-aged children showed similar safety profiles (Namwanje et al., 2011). However, in the current study, inflammation of the body and the face was observed in four children (2.5%). Interestingly, these children were *Schistosoma* egg-negative at the baseline survey before drug administration and only one child showed a light positive POC-CCA result (1+). Inflammation of body and face has been observed in previous studies (Mutapi et al., 2011), raising concern with regard to the inclusion of preschool-aged children in preventive chemotherapy campaigns, which has been proposed by different authors (Bosompem et al., 2004; Stothard et al., 2011). While preschool-aged children with a confirmed *Schistosoma* infection must be treated (Johansen et al., 2007), we feel that further research is still required, including development of an appropriate pediatric formulation, dose-finding, detailed pharmacokinetic investigations, and in-depth safety studies, before preventive chemotherapy be extended from the school-aged population to preschoolers (Keiser et al., 2011).

6.7. Conclusion

In conclusion, our study documents that preschool-aged children are at risk of schistosomiasis in the Azaguié area, south Côte d'Ivoire, with 7% of our per-protocol population patently infected with *S. mansoni* and *S. haematobium* concurrently. Crushed praziquantel is

efficacious against both species in preschool-aged children. In view of unwanted adverse events in non-infected children following praziquantel administration, we suggest that only parasitologically confirmed preschool-aged children should be given praziquantel. New research is needed to accurately determine the frequency and severity of adverse events after praziquantel administration against schistosomiasis in the preschool-aged population. There is a need to develop a safe and user-friendly formulation of praziquantel so that infected children can be treated at an early stage of infection in order to prevent any harmful damage in later life.

6.8. Acknowledgments

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7. Discussion

This Ph.D. thesis focuses on the epidemiology, diagnosis and control of schistosomiasis, placing particular emphasis on preschool-aged children in Azaguié district, southern Côte d'Ivoire (chapters, 9-13). In a first step, we determined the prevalence and intensity of schistosome, soil-transmitted helminths and intestinal protozoa infections in schoolchildren in selected villages of Azaguié district. We explored the effect of sampling effort on the measured prevalence and intensity of helminths infections in four locations of Azaguié district; Azaguié Makouguié and Azaguié M'Bromé (rural), Abbé-Begnini (peri-urban) and Azaguié Gare (urban). Next, we assessed the accuracy of a urine commercially available circulating cathodic antigen (CCA) test for the diagnosis of *S. mansoni* in low (10-24%), and moderate endemicity area (25-49%) and in a setting co-endemic for *S. mansoni* and *S. haematobium*. Moreover, for the purpose of broader validation, the same urine CCA test was validated for the diagnosis of *S. mansoni* in preschool-aged children before and after praziquantel administration. This investigation provided an opportunity to assess the efficacy and safety of crushed praziquantel tablets given to preschool-aged children in a co-endemic setting of *S. mansoni* and *S. haematobium*. Last but not least, we studied the epidemiology of schistosomiasis in preschool-aged children, including focus group discussions and questionnaire addressed to children's mothers or guardians.

Table 7.1 summarises the specific objectives of the present Ph.D. thesis, grouped according to the strategic axes guiding the Swiss Tropical and Public Health Institute (Swiss TPH) research, training and services activities – innovation, validation and application.

The findings of the different cross-sectional surveys, including an intervention study, carried out in the frame of this Ph.D. thesis in epidemiology will be summarized and discussed in the following sections. Future research needs and implications for potential integration of preschool-aged children into schistosomiasis control programmes are highlighted. Our findings might be relevant for the individual patient management level as well as for community-based control programmes in Côte d'Ivoire and elsewhere in sub-Saharan Africa.

Table 7.1: Contribution of the different chapters of this Ph.D thesis to the nexus of Swiss TPH, innovation, validation and application.

Chapter	Title	Innovation	Validation	Application
2	Intestinal parasitic infections in schoolchildren in different settings of Côte d'Ivoire: effect of diagnostic approach and implications for control			Small-scale heterogeneity of intestinal parasitic infections determined in Azaguié district
3	Accuracy of urine CCA test for <i>S. mansoni</i> diagnosis in different endemicity settings of south Côte d'Ivoire		Urine CCA test accuracy for the diagnosis of <i>S. mansoni</i> in school-aged children determined in different endemicity settings	
4	Accuracy of urine circulating cathodic antigen assay for <i>Schistosoma mansoni</i> diagnosis in preschool-aged children before and after treatment		CCA test accuracy for the diagnosis of <i>S. mansoni</i> was assessed in preschool-aged children	
5	Epidemiology of schistosomiasis in two high-risk communities of south Côte d'Ivoire with particular emphasis on pre-school-aged children			Epidemiology and risk factors for schistosomiasis were assessed in preschool-aged children for first time in Côte d'Ivoire
6	Efficacy and safety of praziquantel in preschool-aged children in a co-endemic area of <i>Schistosoma mansoni</i> and <i>S. haematobium</i>		Efficacy of crushed praziquantel in preschool-aged children was assessed for the first in Côte d'Ivoire	

7.1. Intestinal parasitic infections in Azaguié district: implication for diagnosis and control

Implementation of intestinal parasitic infections control programmes require information on the spatial distribution, prevalence and intensities of infections to target interventions mass treatment to areas of greatest need. Identifying these communities requires reliable information on the geographical distribution of infection (Sturrock et al., 2010). To address this operational requirement, there has been increased interest in investigating scientifically robust, yet practical approaches to mapping (Hay, 2000; Tanser and Le Sueur, 2002; Yang et al., 2005; Baker et al., 2010). It has been demonstrated that socio-economic and climate-based risk models can reliably define the large-scale limits of transmission (Brooker et al., 2004; Raso et al., 2005; Brooker et al., 2006; Raso et al., 2006), providing an initial stage in the geographic targeting of treatment. Within these broad limits, epidemiological surveys are still required to identify localities requiring preventive chemotherapy and others intervention. However, large-scale intestinal parasitic infections surveys increase the cost of any programme and may prove difficult for many developing countries to implement because of a lack of technical and financial resources (Mathieu et al., 2003; Kurowski et al., 2007). To reduce programme costs, surveys to guide such targeting should be reliable, yet of lowest possible cost (Brooker et al., 2009). In response to these considerations, efforts have been made to reduce the cost and complexity of surveys for targeting preventive chemotherapy and other interventions by developing survey tools and methods that enable rapid assessment of health problems, such as self-reported blood in urine using school questionnaires and lot quality assurance sampling (LQAS) (Lengeler et al., 2002; Brooker et al., 2005; Steinmann et al., 2010).

Small-scale heterogeneity has important consequences for surveys and controls because it determines the resolution at which surveys and interventions need to be carried out. Diseases that are widespread and evenly distributed (e.g., soil-transmitted helminthiasis) require fewer survey locations than more focal diseases (e.g., schistosomiasis) that require higher resolution data to avoid missing important foci of infection (Utzinger et al., 2003; Clennon et al., 2004; Saathoff et al., 2005).

Defining an optimal sampling scheme for targeting intestinal parasitic infections control requires an understanding of the following issues: (i) the degree of spatial heterogeneity of intestinal parasitic infections; (ii) the financial and human cost of conducting epidemiological

surveys for intestinal parasitic infections; (iii) the geographical framework within which public health decision-making is organized through community, sub-district, district and local levels; and (iv) the financial and public health consequences of inappropriate control decision on the need for preventive chemotherapy and other control measures (Sturrock et al., 2010).

Between October and November 2010, we pursued a cross-sectional epidemiological survey to characterize *S. mansoni*, *S. haematobium*, soil-transmitted helminth and intestinal protozoa infections in school-aged children in three settings (rural, peri-urban and urban) located within a radius of 12 km in Azaguié district, south Côte d'Ivoire. One hundred and seventy, 146, and 130 schoolchildren were enrolled in the rural, peri-urban and urban settings, respectively. We found *S. mansoni* prevalence of 91.8%, 32.9% and 53.1% in the rural, peri-urban and urban settings, respectively. The prevalence of *S. haematobium* was 65.3%, in the rural setting, whilst in the peri-urban setting and urban setting, prevalence were much lower, 4.1% and 0.8%, respectively. Three issues are worth highlighting. First, *S. mansoni* is highly endemic in the Azaguié district, confirming previous results (Matthys et al., 2006b). Second, *S. haematobium* shows a more focal distribution in the current settings. Third, we found a higher *S. mansoni* prevalence in the urban compared to the peri-urban setting. The latter observation confirms finding from Man region in western Côte d'Ivoire (Matthys et al., 2006a). Hence, schistosomiasis is not only a rural disease. Urbanisation seems to be a problem. The occurrence of *S. mansoni* in urban setting might be due to the development of built-up areas, which are characterized by disorganized town-planning (Gryseels and Ngimbi, 1983; Meunier et al., 1984; Sarda et al., 1985; Soares et al., 1995; Utzinger and Keiser, 2006). The poor management of human waste in this context leads to high faecal pollution of the environment, causing a contamination of the urban hydrographic network. Moreover, agricultural activity, including irrigated rice farming occurs within many medium-sized towns in developing countries, particularly in Côte d'Ivoire (Matthys et al., 2006b; Matthys et al., 2006a) managed urbanization pose considerable public health challenges.

Ours findings are important for decision-makers and urban planners while previous studies already emphasized that Azaguié is an important focus of schistosomiasis (N'guessan et al., 2006; Coulibaly et al., 2011), we now present detailed small-scale heterogeneity not only of *S. mansoni* and *S. haematobium*, but also of others intestinal parasites and multiparasitism at this sub-prefecture level. To enhance efficiency, control programme should consider the small-scale heterogeneity of schistosomiasis, soil-transmitted helminths, and intestinal protozoa infections guiding integrated control measures.

7.2. Need for accurate diagnostic assays in the era of preventive chemotherapy

In spite of the prolific generation of new knowledge in the area of schistosomiasis, there are some unsolved practical issues associated with the diagnosis and control of this parasitic disease. This is particularly true in relation with the laboratory diagnosis of this trematode infection.

In areas of low transmission or in individuals who have received preventive chemotherapy, infections are mainly of light intensity (Utzinger et al., 2001; Knopp et al., 2011). It is important to note that in many countries in Africa, schistosomiasis control programmes have gone to scale, and hence, prevalence, intensity and morbidity have been greatly reduced (Zhang et al., 2007; Toure et al., 2008; Fenwick et al., 2009). The future challenge for control programmes that are now moving to elimination is to detect lightly infected individuals (Rollinson et al., 2012).

The widely used Kato-Katz technique for the diagnosis of *S. mansoni* (and soil-transmitted helminths) lacks sensitivity, particularly in low endemicity areas and after anthelmintic drug administration (Utzinger et al., 2001; Booth et al., 2003; Enk et al., 2008; Knopp et al., 2008; Utzinger et al., 2011). Hence, there is a need for a diagnostic tool that is accurate, rapid and field applicable. Recent studies have shown that indirect diagnostic tests (e.g., point-of-contact circulating cathodic antigen (POC-CCA)) have become valuable alternatives to direct parasitological methods for the diagnosis of *S. mansoni* (Coulibaly et al., 2011; Shane et al., 2011; Stothard et al., 2011b).

We conducted two cross-sectional studies, first in October November 2010 and August September 2011 in Azaguié district, south Côte d'Ivoire. The first study focussed on schoolchildren, the second on preschool-aged children. The aim of the two studies was to assess the accuracy of a urine CCA test. In both study populations we found that a single urine CCA test was more sensitive than multiple Kato-Katz thick smears (Coulibaly et al., 2011; Coulibaly et al., 2012). Despite the good performance of this urine CCA test in schoolchildren as well as preschool-aged children, limitations of our studies and the CCA test need to be highlighted. First, due to the lack of sensitivity of the Kato-Katz technique in low infection intensity settings, we have no true 'gold' standard. As with our previous studies we therefore combined results from several diagnostic methods in an attempt to come close to a 'gold' standard (Bogoch et al., 2006; Knopp et al., 2008; Steinmann et al., 2008). This approach allowed us to assess the accuracy of our CCA test. It follows that the accuracy of the urine CCA test might be over-estimated.

Second, the CCA test can only detect one parasite species, namely *S. mansoni*. The Kato-Katz techniques, on the other hand can detect intestinal parasites (e.g., *S. mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Enterobius vermicularis*). It is well-established that in most of the tropical endemic settings, polyparasitism is the norm rather than the exception (Brooker et al., 2000; Keiser et al., 2002; Raso et al., 2004; Steinmann et al., 2010). The development of multiplex real-time PCR might offer a way forward as sensitivity is high and diagnosing multiple parasites concurrently is a major asset (Liu et al., 2008). However, until multiplex real-time PCR becomes available in the field, much remain to be done.

Third, after successful treatment, individuals might still release *S. mansoni* antigens in their urine for several weeks (van Lieshout et al., 1994; Nibbeling et al., 1998; Legesse and Erko, 2007). At the individual patient management level this could lead to a misclassification of healthy people, and hence a waste of drugs.

Despite these aforementioned limitations of the urine CCA test validated here, we feel that it is a useful tool for point-of-contact diagnosis and for the monitoring of schistosomiasis control programmes. It is much more sensitive than widely used Kato-Katz technique, can be used with ease even by a laymen does not require electricity, and hence can be used in remote areas where microscopes are unavailable. Taken together, these issues highlighted a urine CCA test indeed plays an important role for the diagnosis of *S. mansoni*.

7.3. Schistosomiasis in the preschool-aged child

Schistosomiasis remains of considerable public-health importance in sub-Saharan Africa (King et al., 2005; Gryseels et al., 2006; Hotez et al., 2006b; Steinmann et al., 2006; Utzinger et al., 2009; Schur et al., 2011). School-aged children are at highest risk of infection and morbidity (Jordan and Webbe, 1993). By focusing treatment upon the school-aged population, WHA resolution 54.19 neglects children of pre-school age, thus preventing them from potential benefits from praziquantel-based preventive chemotherapy campaigns, which creates a potential health inequity (Johansen et al., 2007). Root causes include the belief that very young children would not yet been exposed to infested freshwater bodies, an insufficient understanding of the epidemiology of schistosomiasis in this age class, and a paucity of pharmacokinetic safety data of praziquantel among children below the age of 4-5 years (Allen et al., 2002; Geary et al., 2010). However, several studies have shown that children before

they reach their fourth or fifth birthday are at substantial risk of urogenital and intestinal schistosomiasis. (Bosompem et al., 2004; Ekpo et al., 2010; Garba et al., 2010; Dabo et al., 2011). Moreover, early infections are most likely not acquired by active water contact of the young children themselves. Instead, the passive exposures to infected water, owing to the bathing and water-drawing practices of children's mothers/guardians puts the youngest children at risk of infection and negative health consequences (Odogwu et al., 2006; Stothard et al., 2011a). Garba et al. (2010), in a study conducted in the Niger River basin highlighted a particularly disturbing situation, as very young children were co-infected with *S. mansoni* and *S. haematobium*. This observation on co-infection raises another avenue of research, detailing morbidity patterns in young children to *S. haematobium* and *S. mansoni* separately and a concurrent infection with both species.

Our cross-sectional study carried out in August/September 2011 to assess the epidemiology and risk factors for schistosomiasis in preschool-aged children in two villages of Azaguié district, revealed a prevalence of 25.5% and 21.6 for *S. mansoni* in Azaguié Makouguié and Azaguié M'Bromé, respectively. The respective prevalence of *S. haematobium* was 17.3% and 5.9%. Coinfection with *S. mansoni* and *S. haematobium* was 10.9% and 5.2%, respectively, considerably higher than what would have been expected by chance (assuming independent distribution of the two species; 1.0-5.5%). However, based on a more sensitive test (urine CCA) for the diagnosis of *S. mansoni*, the prevalence was 44.0% in Azaguié Makouguié and 45.9% in Azaguié M'Bromé. The youngest age at which an infection with *S. mansoni*, based on the Kato-Katz or a urine CCA test, was 8 months and 3 months, respectively. Most infections were of light intensity. Taken together our findings call for a consideration of schistosomiasis in the preschool-aged child as a public health issue at least from the following standpoints. First, the preschool-aged children might play a subtle role in maintaining local disease transmission. Even though these infected children may be excreting fewer eggs, it is their regular contact with water that leads to environmental contamination. Moreover, as discussed by Stothard and Gabrielli, (2007) and others, rinsing and washing children's clothes in environmental water bodies contribute to further contamination and disease transmission (Sousa-Figueiredo et al., 2010b; Stothard et al., 2011b). Second, regular water contact (often forced by mothers/guardians) is likely to result in frequent re-infections, and hence accumulation of individual worm burden (Stothard et al., 2011b).

Côte d'Ivoire has witnessed several years of socio-political problems and the national schistosomiasis control programme has struggled to properly take off. In many settings, school-aged children and other community members had no access to treatment and other control measures. It is therefore conceivable that, for many years, in the absence of treatment infections have accumulated, which might explain the relatively high infection prevalence we observed already in the preschool-aged children. It remains to be determined whether these young children have pathological lesion. Chronic *S. haematobium* can cause bladder wall pathology, leading to ulcer formation, haematuria and dysuria. Granulomatous changes and ulcers of the bladder wall and ureter can lead to bladder obstruction, dilatation and subsequent bladder calcification and renal failure. Pathologies due to chronic *S. mansoni* infection includes lesion of the liver, portal vein, and spleen, leading to periportal fibrosis, portal hypertension, hepatosplenomegaly, splenomegaly, and ascite (Hatz, 2001; Hotez et al., 2006a; Lambertucci et al., 2008).

Concluding, our results and those from others, indicate that schistosomiasis is a public health issue among preschool-aged children in Azaguié district. Overlooking this fact could jeopardize efforts of control programmes. However, further investigation to deepen our understanding of the epidemiology, risk factors and idiosyncracies of socio-ecological systems are needed.

7.4. Efficacy and safety of praziquantel in preschool-aged children

The anthelmintic drug praziquantel is the cornerstone of schistosomiasis control (WHO, 2002; Fenwick et al., 2003; Doenhoff et al., 2008). Indeed, since the mid-1980s, schistosomiasis control emphasizes morbidity control (WHO, 1985), which has been reinforced by WHA resolution 54.19 encouraging member states to regularly treat at least 75% and up to 100% of school-aged children at risk of schistosomiasis and soil-transmitted helminthiasis (WHO, 2002; Savioli et al., 2009). Preschool-aged children (<6 years) are currently excluded from preventive chemotherapy as discussed before. There is only limited documentation on the safety of praziquantel in this age group. To fill this knowledge gap, WHO supported treatment trials in Mali, Sudan and Zimbabwe. The aim was to assess the efficacy and safety of crushed praziquantel tablet or as a suspension in preschool-aged children. These studies revealed high cure and egg reduction rates of praziquantel (either tablet formulation or suspension) (WHO, 2010). It should be noted, however that most studies

on preschool-aged children used crushed praziquantel (Garba et al., 2010; Stothard et al., 2011a), as praziquantel in suspension formulation is only available in Egypt.

We designed a clinical trial to assess the efficacy and safety of crushed praziquantel in preschool-aged children. The study was implemented between August and November 2011. We found high cure against *S. mansoni* 88.6% and *S. haematobium* 88.9% and egg reduction rates (96.7% and 98.0%), respectively. Our findings confirm that crushed praziquantel administered to preschool-aged children is highly efficacious (Garba et al., 2010; Stothard et al., 2011a). With regard to safety, we observed four children (2.5%) with face and body inflammation within the first four hours of praziquantel treatment. This adverse event required the presence of medical doctor to advice children's mothers/guardian. Interestingly, these four adverse events were observed among children without an infection with *Schistosoma*. Hence the risk at benefits of praziquantel administration in this age group must be better understood before any policy recommendations.

In conclusion praziquantel his highly efficacious against *S. mansoni* and *S. haematobium* in preschool-aged children. Despite the high efficacy of crushed praziquantel tablets in this age group, the observed adverse events are of concern. While efforts should be made to avoid the health inequity evoked by some authors, safety is crucial (Johansen et al., 2007). New research is needed and we call for a standard approach for the evaluation of praziquantel in preschool-aged children. This approach should make comparable the studies and the outcomes they produce, but also document the efficacy and safety of praziquantel in this age group.

7.5. Integration of preschool-aged children in preventive chemotherapy: challenges and opportunities

There is a growing awareness among donors and the scientific community about schistosomiasis as a public health problem among preschool-aged children (WHO, 2010; Ekpo et al., 2012). In the past decade, efforts have been made to document the epidemiology of schistosomiasis in different social-ecological settings, including appraisal of the efficacy and safety of praziquantel in preschool-aged children, the current treatment of choice against schistosomiasis and many other trematode infections (Utzinger and Keiser, 2004; Keiser and Utzinger, 2009). However, despite these efforts, the decision whether or not to includ

preschool-aged children into large-scale treatment campaign will depend on the local social-ecological settings and requiring that some challenges being overcome.

First, the current literature does not provide any accurate estimation of the number of preschool-aged children at risk of schistosomiasis or the actual number of infections. This is in stark contrast to the situation of malaria (with approximately 86% of malaria deaths globally in the age <5 years) (WMR, 2011; Bryce et al., 2005), HIV/AIDS (Bryce et al., 2005) and soil-transmitted helminths (de Silva et al., 2003). This raises the issue of diagnostic tools. The current widely used Kato-Katz technique fails to detect *S. mansoni* infections in preschool-aged children as they are mainly of low infection intensity. The urine CCA test employed in our work holds promise and warrants further investigations, along with other immunodiagnostic methods and molecular (DNA) tests, such as real-time PCR.

Second, due to the fact that preschool-aged children are often passively exposed to contaminated water, efforts should be made to develop and disseminate setting-specific educational tools and normative behavior change to alter risk profiles. It is encouraging that schistosomiasis elimination efforts launched in Zanzibar have component of normative behavioral changes and lesson learned in Zanzibar might become applicable for other schistosomiasis endemic settings.

Third, most studies carried out thus in preschool-aged children, treatment was based on crushed praziquantel tablets, administered based on children's weight. In addition to the fact that crushed praziquantel administration is time consuming, in endemic settings where resources are limited, weight scales are not often available (Sousa-Figueiredo et al., 2010b). The dose pole designed and proposed by WHO for the treatment of individuals with a height of 94 cm and above could be adapted to preschool-aged children. Recently, calls have been made to extend this dose pole in order to include preschool-aged children in control programme (Sousa-Figueiredo et al., 2010b; Sousa-Figueiredo et al., 2010a; Stothard et al., 2011a). The same procedures for the validation of the current dose pole by WHO could be followed to design a new dose pole including preschool-aged children (Montresor et al., 2001). Otherwise, if treatment of preschool-aged children continues to rely on praziquantel tablets, it is desirable for practical reasons to better manufacture the 600 mg tablets so that they become more easily splittable in four sections instead of two sections as currently. Minitablets might be the way forward, as is currently explored by Marck Sereno.

Fourth, the size of praziquantel tablets and their palatability make them inappropriate for the treatment of preschool-aged children. Hence, a new formulation of praziquantel is

needed. A syrup formulation proposed by WHO has been assessed recently in Mali, Sudan, and Zimbabwe and showed high cure and egg reduction rates against both *S. mansoni* and *S. haematobium* (WHO, 2010). Efforts should be made in this direction. However, further investigations are needed to assess the cost-effectiveness of the syrup formulation in term of logistic, conservation and operational issues, which are crucial if this revised tool should be deployed at large scale.

Fifth, further investigation are needed to continuously assess the efficacy and safety of praziquantel in preschool-aged children. Up to now, nothing is known about the impact of the long-term use of praziquantel on the health of preschool-aged children. Due to their young age and their immune system not yet fully developed, the repeated treatments need to be monitored and the appropriate frequency of the treatment at the local level determined.

Sixth, there is need for interaction of schistosomiasis control activities into wider child health programmes such as, other neglected tropical diseases (e.g., soil-transmitted helminthiasis, malaria and vaccination programmes (Bergquist et al., 2005; Hotez et al., 2006b). This will not only be cost-effective but also mitigate the reluctance of mothers due to pressure from various programmes.

7.6. Conclusion

The overarching goal of this Ph.D. thesis was to deepen our understanding of the epidemiology of schistosomiasis in preschool-aged. Particular emphasis was placed on diagnostic methods and the efficacy and safety of the current treatment of choice. We used three different diagnostic methods, two widely used parasitological techniques (Kato-Katz thick smear for *S. mansoni* and urine filtration for *S. haematobium*) and one immunodiagnostic test (i.e., urine CCA for *S. mansoni*).

Azagué district, located in south Côte d'Ivoire, has been characterized in term of intestinal parasitic infections among school-aged children. Based on these results, the accuracy of urine CCA test was assessed in schoolchildren and subsequently in preschool-aged children. We then pursued a cross-sectional parasitological and questionnaire survey to study the epidemiology and risk factors associated with schistosomiasis in preschool-aged children. Schistosome-infected children were treated with crushed praziquantel tablets, which allowed to assess the efficacy and safety of this treatment regimen in this age group. Based on the result of the work presented in this thesis, the following conclusion can be drawn:

- The heterogeneity of schistosomiasis, soil-transmitted helminthiasis and intestinal protozoa infections at a small-scale should be considered in Azaguié district now that control activities are moving forward. Since polyparasitism was common, an integrated approach is recommended to address multiple neglected tropical diseases (i.e., schistosomiasis, soil-transmitted helminthiasis and pathogenic intestinal protozoa) simultaneously.
- The commercially available urine CCA test is a useful tool for the diagnosis of *S. mansoni* and should be considered for point-of-contact and might become a useful tool in the national schistosomiasis control programme in Côte d'Ivoire to identify high-risk communities and for monitoring purposes. However, further investigations are needed to clarify its use as a tool for the evaluation of drug efficacy studies.
- Preschool-aged are at risk of schistosomiasis in Azaguié district. The exposure of the youngest children <2 years is due to the behaviour of their mothers and caregivers as well as their older siblings. The improvement of safe water supply and sanitation facilities combined with health education could significantly reduce the burden of helminths and intestinal protozoa infections in this district.
- Crushed praziquantel is efficacious against both *S. mansoni* and *S. haematobium* infections in preschool-aged children. However, further investigations are needed to better understand adverse events due to praziquantel treatment in this age group.

7.7. Recommendations

- Study schistosomiasis at the national level and quantify the importance of the disease in preschool-aged children, including morbidity. Particular emphasis should be placed on the population living in close proximity to water resource development and management projects (e.g., large dams and irrigation system).
- Pursue malacological studies to identify the main snail intermediate hosts in our study area and determine the dynamics of schistosomiasis transmission over time. This is useful to better understand the factors linked to schistosomiasis transmission in our study settings and will help to optimize the best timing for treatment and others control interventions.
- Assess the integration of preschool-aged children in schistosomiasis control programme strategies in Côte d'Ivoire in term of cost-effectiveness. That mean that the number of preschool-aged children at risk of schistosomiasis at the national level is well-estimated and the high risk areas determined.

- As long as appropriate formulation for treating preschool-aged children are available, use crushed praziquantel as it is highly efficacious. However alternative formulation for treatment of preschool-aged children must be pursued and the safety monitored closely. Perhaps selective treatment (infected preschool-aged children only) is more suitable than preventive chemotherapy for this age group.
- Establish an integrated approach for schistosomias, soil-transmitted helminths and other neglected tropical diseases that is readily adapted to the local disease spectrum and socio-ecological settings.
- Introduce urine CCA test as a point-of-contact diagnostic tool for intestinal schistosomiasis which will strengthen health system.

7.8. References

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8. Curriculum vitae

Personal data

Full name	Jean Tenena Coulibaly
Place, date of birth	Koumbala, 29.06.1978
Marital status	Single
Nationality	Ivorian
Languages	French (very good), English (good)
Address (work)	Swiss Tropical and Public Health Institute Department of Public Health and Epidemiology Socinstrasse 57 4051 Basel, Switzerland
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Education and work experience

09/2009 - 06/2012	Ph.D in Epidemiology Swiss Tropical and Public Health Institute Department of Public Health and Epidemiology
Ph.D thesis	Epidemiology and diagnosis of schistosomiasis in preschool-aged children in Azaguié, south Côte d'Ivoire
Supervision	Prof. Dr. Marcel Tanner (Swiss TPH), Prof. Dr. Jürg Utzinger (Swiss TPH), Prof. Dr. Eliézer Kouakou N'Goran (Université de Cocody, Côte d'Ivoire), Prof. Dr. Piero Olliaro (WHO/TDR)
06.2012 (3 days)	Meeting to advise on the evaluation of praziquantel efficacy and on setting up programmes to evaluate schistosomiasis diagnostic, WHO, Geneva, Switzerland
03.2011 (2 days)	Initiation on the real-time PCR for the diagnosis of <i>S. mansoni</i> Biolytix laboratory, Switzerland
01.2010 – 10.2010	Support of 3 master students in the implementation of their field work on Neglected Tropical Diseases (NTDs) Agboville and Azaguié, Côte d'Ivoire
09.2009 – 12.2009	Courses on, concepts and methods in Epidemiology; Biostatistic I University of Basel, Switzerland
10.2006 – 12. 2007	M.Sc (Diploma) in Entomology Université de Cocody, Côte d'Ivoire
M.Sc thesis	Inventaire et impact des insectes locaux sur les végétaux aquatiques envahissants: cas de <i>Eichhornia crassipes</i> (Mart) Solm-Laub (Pontederiaceae) et de <i>Salvinia molesta</i> Mitchell (Salviniaceae) dans la commune de Port-Bouët (Abidjan, Côte d'Ivoire)
Supervision	Université de Cocody, Côte d'Ivoire Prof. Dr. Tano Yao (Université de Cocody, Côte d'Ivoire), Prof. Dr. Philippe Kouassi Kouassi (Université de Cocody, Côte d'Ivoire), Prof. Dr. Mathieu Wadja Egnankou (Université de Cocody, Côte d'Ivoire)
10.2006 (one week)	Insects capturing techniques at the Station of Ecology and Geophysics LAMTO, Côte d'Ivoire.
10.2005 – 06.2009	Teacher of life and earth sciences in a private school

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CSM de Cocody, Abidjan, Côte d'Ivoire
Gymnasium Korhogo, Côte d'Ivoire

9. Publications

Müller, I., **Coulibaly, J.T.**, Fürst, T., Knopp, S., Hattendorf, J., Krauth, S.J., Stete, K., Righetti, A.A., Glinz, D., Yao, A.K., Pühse, U., N’Goran, E.K., Utzinger, J. (2011) Effect of schistosomiasis and soil-transmitted helminths infections on physical fitness of school children in Côte d’Ivoire. *PLoS Negl Trop Dis* (5): e1239.

Fürst, T., Müller, I., **Coulibaly, J.T.**, Yao, A.K., Utzinger, J., N’Goran, E.K. (2011) Questionnaire-based approach to assess schoolchildren’s physical fitness and its potential role in exploring the putative impact of helminths and *Plasmodium* spp. infections in Côte d’Ivoire. *Parasit Vectors* 4: 116.

Coulibaly, J.T., Knopp, S., N’Guessan, N.A., Silué, K.D., Fürst, T., Lohourignon, L.K., Brou, J.K., N’Gbesso, Y.K., Vounatsou, P., N’Goran, E.K., Utzinger, J. (2011) Accuracy of circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d’Ivoire. *PLoS Negl Trop Dis* (5): e1384.

Coulibaly, J. T., Fürst, T., Silué, K. D., Knopp, S., Hauri, D., Ouattara, M., Utzinger, J. and N’Goran, E. K. (2012) Intestinal parasitic infections in schoolchildren in different settings of Cote d’Ivoire: effect of diagnostic approach and implications for control *Parasit Vectors*, 5: 135.

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Krauth, S.J, **Coulibaly, J.T.**, Knopp, S., Traoré, M., N’Goran, E.K., Utzinger, J. (2012): An in-depth analysis of a piece of shit: spatial and temporal distribution of helminth eggs. *PLoS Negl Trop Dis* 6 (12): e1969.

Stete, K., Krauth, S.J., **Coulibaly, J.T.**, Knopp, S., Hattendorf, J., Müller, I., Lohourignon, L.K., Kern, W.V., N’Goran, E.K., Utzinger, J. (2012) Dynamics of *Schistosoma haematobium* egg output and associated infection parameters following treatment with praziquantel in school-aged children *Parasit Vectors* 5: 298.

Coulibaly, J.T., N’Gbesso, Y.K., N’Guessan, N.A., Winkler, M.S., N’Goran, E.K., Utzinger, J (2012) Epidemiology of schistosomiasis in two high-risk communities of south Côte d’Ivoire with particular emphasis on pre-school-aged children. *American Journal of Tropical Medicine and Hygiene* (in press).

Coulibaly, J.T., N’Gbesso, Y.K., Knopp, S., N’Guessan, N.A., Silué, K.D., N’Goran, E.K., Utzinger, J. Accuracy of urine circulating cathodic antigen assay for *Schistosoma mansoni* diagnosis in preschool-aged children before and after treatment. *PLoS Negl Trop Dis* (7): e2109

10. Appendix

10.2. Questionnaire based approach to assess risk factors associated with schistosomiasis in preschool-aged children in Azaguié Makouguié and Azaguié M'Bromé. Questionnaire was administered to the mothers/caregivers of preschool-aged children (in French)

Questionnaire socio environnemental

(Questionnaire à administrer à la mère de l'enfant d'âge préscolaire)

Nom de l'enquêteur :

Q3 Village:.....

Date :.....

Q4 Quartier :.....

N° d'ordre enfant :.....

I – ACTIVITE DE LA MERE

Q7 Coché la case qui correspond à votre activité principale pendant la journée.

a	Commerçante	
b	Rizicultrice	
c	Maraîchère	
d	Pêche	

e	Elevage	
f	Artisane	
g	Culture de rente	
h	autre (.....)	

Q8 Vos activités vous contraind-elle à rester souvent dans l'eau ?

1) Non

2) Oui

Q9 Quelle est la durée de votre contact avec l'eau ?

Toute la journée ½ journée Quelques heures Autres (.....)

Q10 Où se trouve votre enfant pendant que vous êtes dans l'eau ?

Au dos Dans l'eau aussi A terre auprès de l'eau A la maison avec ses autres frères/Soeurs

Autres (.....)

II - MIGRATION

Q11 Depuis combien de temps êtes-vous dans ce village? / Année/Mois

Q12 Y a-t-il un membre de votre ménage qui s'absente de la maison pendant plus d'un mois?

1) Non

2) Oui

Q13 a Avez-vous un visiteur chez vous actuellement?

1) Non

2) Oui

Q14 b Si oui d'où vient-il?

Q15 c Depuis combien de temps est-il avec vous? / Année/Mois

III – FOURNITURE EN EAU

Q15 Classer de 1 à 4 les sources de provision en eau de votre ménage de la plus fréquentée à la moins fréquentée

Q15a 1 ^{er} choix		Q15b 2 ^{ème} choix		Q15c 3 ^{ème} choix		Q15d 4 ^{ème} choix	
Fontaine		Fontaine		Fontaine		Fontaine	
Puit traditionnel		Puit traditionnel		Puit traditionnel		Puit traditionnel	
Ruisseau		Ruisseau		Ruisseau		Ruisseau	
Mare		Mare		Mare		Mare	
Eau de robinet		Eau de robinet		Eau de robinet		Eau de robinet	
Rivière		Rivière		Rivière		Rivière	
Marigot		Marigot		Marigot		Marigot	

IV - HYGIENE ET SANTE

Q16 Classer de 1 à 4 les sources d'eau utilisées pour les toilettes de vos enfants de la plus utilisée à la moins utilisée

Q16a 1 ^{er} choix		Q16b 2 ^{ème} choix		Q16c 3 ^{ème} choix		Q16d 4 ^{ème} choix	
Fontaine		Fontaine		Fontaine		Fontaine	
Puit traditionnel		Puit traditionnel		Puit traditionnel		Puit traditionnel	
Ruisseau		Ruisseau		Ruisseau		Ruisseau	
Mare		Mare		Mare		Mare	
Eau de robinet		Eau de robinet		Eau de robinet		Eau de robinet	
Rivière		Rivière		Rivière		Rivière	
Marigot		Marigot		Marigot		Marigot	

Q17 Classer de 1 à 4 les récipients de conditionnement de l'eau dans votre ménage par degré d'utilisation

Q17a 1 ^{er} choix		Q17b 2 ^{ème} choix		Q17c 3 ^{ème} choix		Q17d 4 ^{ème} choix	
Bidon		Bidon		Bidon		Bidon	
cuvette		cuvette		cuvette		cuvette	
Jarree		Jarre		Jarre		Jarre	
Barrique		Barrique		Barrique		Barrique	

Q18 Classer de 1 à 4 les méthodes d'évacuation de vos déchets**Q18a 1^{er} choix**

Dans une poubelle	
Dans un ruisseau	
A côté de la maison	
Maison abandonnée	
Autre.....	

Q18b 2^{ème} choix

Dans une poubelle	
Dans un ruisseau	
A côté de la maison	
Maison abandonnée	
Autre.....	

Q18c 3^{ème} choix

Dans une poubelle	
Dans un ruisseau	
A côté de la maison	
Maison abandonnée	
Autre	

Q18d 4^{ème} choix

Dans une poubelle	
Dans un ruisseau	
A côté de la maison	
Maison abandonnée	
Autre	

Q119 Partagez-vous vos toilettes avec vos enfants?1) Non 2) Oui **Q20 Où vos enfants ont-ils l'habitude de déféquer?**1) Latrine 2) Dans les environ de la maison 3) Dans la cours 4) Dans l'eau de la rivière

5) Autre.....

Q21a Avez-vous l'habitude de laver les mains de votre enfant après qu'il ait déféqué?1) Non 2) Oui **Q21b Si oui, comment?**1) Avec de l'eau savonnée 2) Avec uniquement de l'eau **Q22a Lavez-vous vos mains avant de faire la cuisine?**1) Non 2) Oui **Q22b Si oui, comment?**1) Avec de l'eau savonnée 2) Avec uniquement de l'eau **Q23a Vos enfants lavent-ils leur main avant et après avoir manger?**1) Non 2) Oui **Q23b Si oui, comment ?**1) Avec de l'eau savonnée 2) Avec uniquement de l'eau

V – AIRE DE JEU DES ENFANTS

Q24 Classer de 1 à 4 les aires de jeu de vos enfants selon le degré de préférence

Q24a 1 ^{ème} choix		Q24b 2 ^{ème} choix		Q24c 3 ^{ème} choix		Q24d 4 ^{ème} choix	
Dans les herbes		Dans les herbes		Dans les herbes		Dans les herbes	
Dans les maisons abandonnées		Dans les maisons abandonnées		Dans les maisons abandonnées		Dans les maisons abandonnées	
Au marigot		Au marigot		Au marigot		Au marigot	
A la rivière		A la rivière		A la rivière		A la rivière	
Dans la cours		Dans la cours		Dans la cours		Dans la cours	

VI – ACCES AU SYSTEME DE SANTE

Q25 Y a-t-il au moins un centre de santé dans votre localité?

1) Non 2) Oui Q26 A quelle distance estimée vous le centre de santé le plus proche de votre ménage? / m/Km

Q27 Quel est le moyen de transport le plus fréquemment utilisé pour conduire vos enfants à l'hôpital?

1) A pied 2) A vélo 3) A mobylette 4) En voiture

Q28 Enumérer les moyens de soin que vous choisissez pour vos enfants par degré de fréquentation (1 à 4)

Q24a 1^{er} choix

Guérisseur	
Infirmier du village	
Centre de santé	
Auto-medication	

Q24b 2^{ème} choix

Guérisseur	
Infirmier du village	
Centre de santé	
Auto-medication	

Q24c 3^{ème} choix

Guérisseur	
Infirmier du village	
Centre de santé	
Auto-medication	

Q24d 4^{ème} choix

Guérisseur	
Infirmier du village	
Centre de santé	
Auto-medication	

