Schistosomiasis in Eastern Democratic Republic of the Congo: A major neglected healthcare concern.

Inaugural Dissertation

zur

Erlangung der Würde eines Doktors der Philosophie vorgelegt der

Medizinische Fakultät der Universität Basel

von

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aus Bunia, Democratic Republic of Congo

Basel, 2020

Genehmigt von der Medizinischen Fakultät

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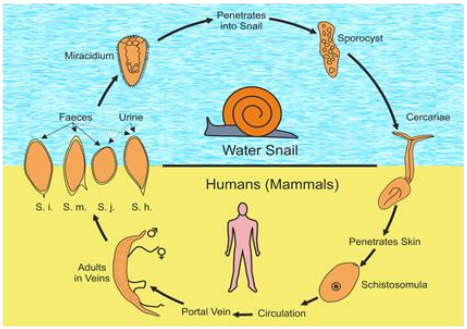
Basel, August 27, 2020

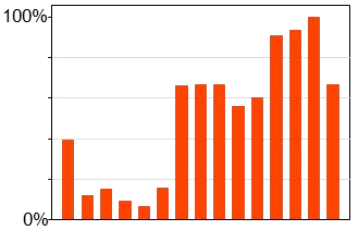
Dekan Prof. Dr. Primo Leo Schär

Schistosomiasis in Eastern Congo: *Graphical Abstract*

S. mansoni egg



Schistosoma spp life cycle



S. mansoni prevalence in school kids 2015

Activities

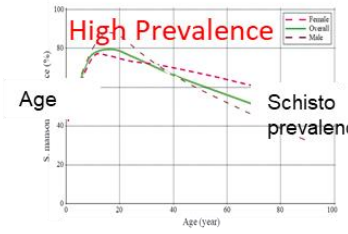
Recognition of undocumented schistosomiasis problem in the area of the Great African War

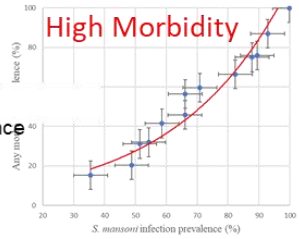
Review of state of the art in schistosomiasis

Is there a problem ?
Planning and performing focused, explorative field study 2015

Scope of the problem ?
Prevalence, geographic extent ?
Planning and performing province-wide large scale field study 2016: 2131 individuals in 46 villages

Morbidity?
Socioeconomic background?
Value of new diagnostics?
Planning and performing in-depth field study 2017: 1022 individuals in 13 villages





Findings & achievements

1) **Unexpectedly high prevalence** in kids & across all ages

2) **Huge challenges for field study execution** in former war zone: roads, infrastructure, security

3) **Map of *S. mansoni* prevalence** of a province of 1.5x the size of Switzerland

4) **Identified** age-related, environmental, socioeconomic, and geographical **risk factors**

5) **Real-world validation of new point-of-care tests**

6) **Determined best-use scenarios** for old and new diagnostics

7) **Quantified** and **significant** attributable morbidity
8) **Severe poverty** as risk factor, and hindering disease control

9) **No discernable** population health **benefit** of **natural resources, industrial & artisanal mining**
10) **Current prevention efforts do not reach the population**

11) **Child health is particular concern**

12) **Major concerted efforts needed** to remedy causal factors, control disease across all ages, reduce related morbidity.

Planning of future large-scale healthcare interventions !

Dedication

This work is dedicated to the people of Ituri province, and its elected leaders.

Acknowledgements

"I will give thanks to you, Lord, with all my heart; I will tell of all your wonderful deeds. I will be glad and rejoice in you; I will sing the praises of your name, O Most High".

This thesis is the product of contribution of individuals and institutions to whom I am deeply indebted and to whom I sincerely acknowledge and express my gratitude. I will need a book to express my gratitude to the many people who have richly and generously contributed to my life. So, I may not list all but I do appreciate every support be it financial, spiritual or intellectual and moral.

Above all, I am indebted to the Almighty God, Master of circumstances and times, for His love, grace and faithfulness. To Him be praise, honour and glory.

I am very grateful to my supervisor Professor Patrick R Hunziker who invested most of all the financial, intellectual and moral support without which thesis would not be possible. Thank you very much, Patrick.

I am indebted to Professor Manuel Battegay and to Professor Peter Odermatt for their support. I would like to put a particular emphasis here on Peter who tirelessly welcomed me and gave many valuable suggestions and advice.

This PhD could have been performed without the support in motivation and workplace through the Intensive Care Units of the University Hospital Basel and kind support by the hospital director, Dr. Werner Kübler, and his management team.

I am indebted for the generous financial support from anonymous private donors, and a very valuable contribution to the costs of a field study by Dr. Andreas Loeffler.

My deepest thanks to Dr. Georgette Salieb-Beugelaar who has invested a lot of her energy, her time and her finances so that this thesis could come to light. Thank you very much, Georgette. I would like to extend my thanks all my colleagues of Nanomedicine Research Group including Marc, Oleksii, Dimytro, Gabor, Kegang, Daniel, Xueya, Bei and Timothy. Thank you for your support.

I am deeply indebted to the Hunziker family for the unforgettable gifts they provided during my stay here in Switzerland. Thank you very much Dear Jack, Maria, Susy, Patrick, Gabi, and Sasha. I promise never to forget your love for me and my family.

I would like to emphasize the love and untold generosity of my dear "Mama" Odette Orsinger. Thank you very much, Odette. Thanks to Professor Albert Adelin of the university of Liège,

Belgium, for contributing valuable advices and sending me the required documents at the beginning of my PhD studies.

I would like to mention here the tremendous contribution of Dr. Montresor, WHO, Geneva, who graciously provided the Kato-Katz kits needed for this study. Thanks to Mr. Messager from "Ultrasound Markt" who supplied a portable ultrasound device for the 2017 study. Many thanks to Dr. Tony Ukety who contributed a lot to the success of my studies. Thanks to Mrs. Yvette Endriss and Christine Mensch of SwissTPH, for contributing valuable study consumables. Thanks a lot to Mr Dehoogh of SwissTPH who made the DRC map and Joe Kabwe who made Ituri map.

This thesis utilized data collected in Ituri province health districts. We gratefully acknowledge the help of Dr. Louis Tsulo, Head of the Ituri Provincial Health Division. We received support from Centre Médical Evangélique (CME) and Institut Supérieur des Techniques Médicales (ISTM), Nyankunde and from our research team: Drs. Nancy and Philip Wood, Dr. Aimé Kiza, Mr Robert Agenong'a, Mr Timothée Kossianza, and others. Dear Francoise Nyisi, Germain Abhafule, Benjamin, Ghislain Amayo, Wully Nigo and many others, thank you very much for your help in field work.

The idea to study this disease in-depth also originated in discussions with Dr. Narcisse Kabatereine, Vector Control Division, Ministry of Health, Uganda to whom I am endebted.

Life would never be good without the great friends in Basel. I am endebted to Riana Paola, my mentor who helped me with her advices. Thanks to Professor Isabelle Charriate Fels, Mrs Hildegund Herles-Schatzmann, Mrs & Mr Albert De Pury, Mr Peter Leuenberger, Pastor Jacques Weber, Pastor David Manduka and members of the congregation of Basel Baptist Church, Adullam, and all my neighbours at Birsfelden.

I thank and acknowledge those who have assisted me my family when I was away from home. My thanks to Anne-Christine and Jeff Horton, Yvonne Dind, Dr Nancy and Philip Wood, Dr Aimé Kiza, Robert Agenong'a, all members of CME Board and the elders of CE-39 Church.

Lastly, to my wife Christine Azonye Atay, our children, our grandchildren and Nigo family siblings including Samy Lemery and Rose, Esther Moseka and David, David Wully and Nancy, Dieu-Donné Alege, Patrick Ayiko, Héritier Ayile, Trésor Ang'ume, Jonathan Aunyi, Emmanuel Iyete, Ruth Dhiria, Akulia Bonebana and others for holding on me during my absence from home to this PhD. Know that there is "no rose without thorn" and "no pain no gain". Thank you for your patience and your faithfulness. I appreciate for your giving and I love you.

Content

Summary

Among the neglected tropical diseases (NTDs), schistosomiasis remains a major public health issue in sub-Saharan Africa (SSA). Schistosomiasis is a blood fluke parasitic infection, caused by several species of trematodes of the genus *Schistosoma*. In sub-Saharan Africa, urinary schistosomiasis is caused by *S. haematobium*, whereas intestinal schistosomiasis is caused by *S. mansoni*. It is transmitted during contact with water contacts while fishing, washing, bathing or swimming. The infective larvae released by the freshwater snail as the intermediate host, penetrate through the skin, which are then transported by the blood, and become adults in the targeted blood vessels. Adult parasites lay eggs in the blood vessels, some of which are trapped in the tissues and are the root of the pathology. Recurrent and massive infections can primarily affect the liver and cause various chronic signs and symptoms. The resulting morbidity can be significant. Symptoms of schistosome infection vary from simple skin rashes to severe blood vomiting. Chronic schistosomiasis happens when diagnosis has not been performed quickly enough during disease progression. However, a large proportion of the infected population has no symptoms, and therefore a significant number of individuals may never be evaluated for infection burden, and so remains as a reservoir of the parasites and indirect infection source to others.

The current diagnosis is mainly based on clinical symptoms, which results in missing the identification of low level and asymptomatic/atypical or chronic infections. Undetected and untreated infections may be responsible for persistence of transmission. Rapid and accurate diagnosis is the key for treatment and control. Various diagnostic procedures are available; validated and include immunologic methods, direct parasitological techniques and molecular approaches. So far, parasitological detection methods remain the cornerstone of schistosoma infection diagnosis in endemic regions but conventional tests have limited sensitivity, in particular in low-grade infection. Recent advances contribute to improved detection in clinical and in field settings. The recent progress in micro- and nano- technologies opens a road by enabling the design of new miniaturized point-of-care devices and analytical platforms, which can be used for rapid detection of these infections. There is still a need for new diagnostic tests at the point-of-care (POC) in the endemic areas of low-income countries which would enable effective treatment and disease management.

In 2001, the World Health Assembly (WHA) resolution WHA 54.19 recommended the reduction of morbidity due to schistosomiasis. Since then, number of control programs in many countries have been established and started to treat more than 75% of school-age children in the endemic areas. Although these programs had obtained mixed successes, several countries in the region have implemented the recommendations of the resolution. Meanwhile, the Democratic Republic of Congo (DRC), one of the most affected countries, entangled in endless multiform conflicts and permanent instability, could not implement a viable control program to address schistosomiasis issue. In eastern DR Congo for the first time since decades, explored morbidity, examined demographic, geographic and socioeconomic factors, including 3 153 patients in 59 villages in an area of 65 658 km². Schistosomiasis prevalence was up to 90%, particularly in children, corresponding to millions of undiagnosed and untreated cases in this region alone, spreading far beyond historic hotspots. Predisposing factors included poverty, lack of good sanitation, limited access to clean water, vegetation characteristics, water contact activities, lack of knowledge on disease transmission and prevention. Significant morbidity was found. Profound poverty was contrasted with the richness in minerals exploited; proximity to mining sites was not associated with reduced poverty or reduced disease prevalence. Infrastructure was severely damaged and security was fragile. Past, national healthcare efforts had left little trace, and current Ebola containment activities nearby drain personnel away from basic healthcare.

In the DRC, the burden of NTDs in general, thus that of schistosomiasis, particularly, remains unknown. There is huge lack of surveys and publications. Most publications date from colonial time. For Ituri province, the most recent publication on schistosomiasis is from 1954. Despite these challenges, DRC started integrated control programs of NTDs including schistosomiasis in 2012, but the implementation did not take place until 2016 in Ituri province.

The main goal of this thesis is to enhance our understanding of the geographical distribution of intestinal schistosomiasis and the environmental, geographical, socioeconomic, and behavioural factors that underlie its transmission and its spread across Ituri province. This knowledge base will advocate for and facilitate the establishment of an effective and sustainable large-scale control and surveillance program for schistosomiasis in the Ituri province.

In this thesis, we have pursued five specific objectives. First, review of the current diagnostic tools available for endemic settings. Second, present the underlying context for the transmission and spread of intestinal schistosomiasis in the Ituri province, DRC. Third, assessthe distribution

of *S. mansoni* infection prevalence and intensity, and determine the main risk factors in the the province. Fourth, assess the associated intestinal and hepatosplenic morbidity. Fifth, describe some severe cases linked with *S. mansoni* infection. For this purpose, three different surveys were carried out, namely (i) a pilot survey among 435 schoolchildren from 7 primary schools for the appraisal of the situation ; (ii) a large community-based survey within 51 villages across the province with 2,131 participants of 1 year and older, both female and male ; and (iii) an in-depth and rigorous household-based survey within 13 villages and with 1,022 participants of 1 year and older, both female and male.

The field investigation activities related to this thesis were conducted in three field campaigns in Ituri province, eastern Democratic Republic of Congo. We first carried out in 2015, a cross-sectional pilot survey involving 435 schoolchildren in seven primary schools in three health districts across the province, in order to analyse the situation. Only one stool sample was requested and examined in accordance to the Kato-Katz (KK) technique. Demographic and anthropometric (weight and height) data were collected and completed in an appropriate form.

Based on the results and needs identified in 2015, we then conducted in 2016 a large, systematic, cross-sectional survey which covered 2,131 participants from 51 villages across the province. This survey aimed to assess the prevalence and intensity of infection within the different categories of the population aged 1 year or older. Once again, weight, height, and demographics were collected and only one stool sample was requested and obtained per participant and examined using KK technique. In addition, an abdominal ultrasound was performed on a limited number of participants. This field campaign resulted in a comprehensive map of schistosomiasis prevalence but also highlighted the need for improved diagnostic tools and in-depth understanding of morbidity.

To address this need, in 2017, we conducted a rigorous in-depth, cross-sectional survey among 1,022 individuals from 145 households in 13 randomly selected villages from the areas where the prevalence was found to be relatively high during the previous surveys. Household characteristics were collected. Household heads or representatives responded to an in-depth household questionnaire about house building material, household income, livestock, objects, access to water, sanitation, presence and quality of latrine, use of the latrine. Each participant responded to an individual questionnaire concerning matrimonial status, occupation, education, religion, drinking water, hand wash, body hygiene, hygiene care of clothes, where they are washing their clothes, where they are bathing, farming, fishing activities, owning shoes,

wearing shoes habits, consumption of alcohol, smoking, length of stay in the village, knowledge of schistosomiasis, its transmission, its prevention, and its treatment, and what they think about the importance of schistosomiasis, whether it is a problem, and the overall attitudes towards it. Only permanent members of the household participated. Parents or household heads responded to some difficult questions of the behalf of their children under six years old. After this, every participant was weighed and its height measured. Participants were asked to provide one stool sample per day for five days in row. On the last day, a urine sample was requested from 75% of participants who had provided at least one stool sample in the period. All stool specimens were examined by the KK technique and the urine was tested by point-of-care circulating cathodic antigen (POC-CCA) test. Approximately, 85% of participants underwent clinical examinations, including abdominal palpation for large liver and large spleen research, observation of conjunctival staining for anaemia, and observation of the skin for rash and scabies, and abdominal percussion to detect ascites. Body temperature was measured in the very few participants who reported current perception of fever. Most participants who underwent clinical examinations also had abdominal ultrasound exams to estimate liver and spleen size, to detect both portal hypertension and fibrosis, to assess gallbladder size, thickness and contents, to detect and classify the types of ascites, and to look for any anomaly of the digestive organs.

All participants through the three successive surveys received 500 mg of mebendazole for general deworming. All individuals found positive for schistosomiasis by either the KK technique or the POC-CCA alone or combined received 60 mg/kg of praziquantel for treatment of the detected schistosoma infection.

In pursuit of the five objectives mentioned above, we structured our results as follows:

1 We provided an update of current relevant achievements in the field of schistosomiasis diagnosis.

2 We documented the challenging context of this disease, specifically wars and ongoing armed conflict, poverty and rush for precious minerals, also involving significant population displacements, that largely promoted the transmission and spread of schistosomiasis in previously unaffected areas of schistosomiasis in the Ituri province and prohibited efforts to detect or control disease. Today, the entire province of Ituri is plagued by the spread of the disease.

3 We described the distribution of *S. mansoni* infection and intensity among socio-demographic and geographic categories. The prevalence is high in the province: in some villages, it reaches 90% or more of those tested, highlighting the existence of relevant undiagnosed disease in a large segment of the population. The prevalence curve by age and sex is typical of high endemic areas where control programs are not yet implemented. The intensity of *S. mansoni* infection is immense. We identified that the main risk factors for schistosomiasis were poverty, the lack of latrine, of adequate sanitation, and of safe water; the surrounding environment that could favour the multiplication fresh-water intermediate host snails, activities in contact with the water such as fishing, washing clothing, dishwashing, farming and also the lack of knowledge about transmission and prevention of schistosomiasis. Other intestinal parasites including roundworms, hookworms, whipworm, tapeworms, and even some cases of *S. intercalatum* infection have also been diagnosed.

4 We provided for the first time since colonial time a comprehensive baseline data showing a high intestinal and hepatosplenic morbidity burden in Ituri province; a burden that is associated with *S. mansoni* infection at both the individual and community level.

5 We reported 8 severe cases from four villages with a very high *S. mansoni* prevalence up to 87.1%. Fifty-six percent of the population was underweight. Intestinal and hepatosplenic morbidity were highly frequent.

In conclusion, **s**chistosomiasis is a major health issue in Ituri province. Prevalence and intensity of infection, and morbidity are high. Several factors including poverty, lack of both latrine, good sanitation, safe water, and education promotes the transmission and spread of schistosomiasis across the province. New diagnostic tools are urgently required to improve diagnosis, case management, mapping, development of control strategies, and monitoring of control programs.

These findings call for concerted efforts in implementation of effective control interventions that may quickly reach the most disadvantaged segments of population in the Ituri province. These interventions should include education of population, improved access to safe water, sanitation and hygiene facilities as well as snail control. The results of these investigations may contribute to the planning of efficient and sustainable control programs, based on revised strategies and can be used by decision-makers in the province of Ituri, eastern Democratic Republic of Congo.

Résumé

Parmi les maladies tropicales négligées (MTN), la schistosomiase reste un problème majeur de santé publique en Afrique subsaharienne (ASS). La schistosomiase est une infection parasitaire de la douve du sang, causée par plusieurs espèces de trématodes du genre *Schistosoma*. En Afrique subsaharienne, la schistosomiase urinaire est causée par *S. haematobium*, tandis que la schistosomiase intestinale est causée par *S. mansoni*. Elle se transmet par contact avec l'eau lors de la pêche, du lavage, de la baignade ou de la natation. Les larves infectantes libérées par l'hôte intermédiaire, l'escargot d'eau douce, pénètrent à travers la peau ; elles sont ensuite transportées par le sang et deviennent adultes dans les vaisseaux sanguins cibles. Les parasites adultes pondent des œufs dans les vaisseaux sanguins, dont certains sont piégés dans les tissus et sont à l'origine de la pathologie. Les infections récurrentes et massives peuvent principalement affecter le foie et provoquer divers signes et symptômes chroniques. La morbidité qui en résulte peut être importante. Les symptômes de l'infection à schistosome varient de simples éruptions cutanées à de graves vomissements sanguins. La schistosomiase chronique survient lorsque le diagnostic n'a pas été posé assez rapidement au cours de l'évolution de la maladie. Cependant, une grande partie de la population infectée ne présente aucun symptôme et, par conséquent, un nombre important d'individus peut ne jamais être évalué pour la charge d'infection, et reste donc un réservoir de parasites et une source indirecte d'infection pour les autres.

Le diagnostic actuel est principalement basé sur les symptômes cliniques, ce qui fait qu'il n'est pas possible d'identifier les infections de faible niveau et/ou asymptomatiques/atypiques ou chroniques. Les infections non détectées et non traitées peuvent être responsables de la persistance de la transmission. Un diagnostic rapide et précis est la clé du traitement et du contrôle. Diverses procédures de diagnostic sont disponibles ; elles sont validées et comprennent des méthodes immunologiques, des techniques parasitologiques directes et des approches moléculaires. Jusqu'à présent, les méthodes de détection parasitologique restent la pierre angulaire du diagnostic des infections à schistosomes dans les régions endémiques, mais les tests conventionnels ont une sensibilité limitée, en particulier pour les infections de faible intensité. Les progrès récents ont contribué à améliorer la détection en clinique et sur terrain. En particulier, les récents progrès dans les micro- et nanotechnologies ont ouvert une voie en permettant la conception de nouveaux dispositifs miniaturisés pour les services de soins et la mise au point de plateformes analytiques qui peuvent être utilisés pour la détection rapide de ces infections. Il existe toujours un besoin de nouveaux tests de diagnostic aux services de soins

dans les zones endémiques des pays à faible revenu ; ce qui permettra un traitement rapie et une gestion efficace des cas.

En 2001, la résolution WHA 54.19 de l'Assemblée mondiale de la santé (AMS) a recommandé la réduction de la morbidité due à la schistosomiase. Depuis lors, de nombreux programmes de lutte ont été mis en place dans de nombreux pays, avec comme objectif le traitement de plus de 75 % des enfants d'âge scolaire dans les zones endémiques. Bien que ces programmes aient obtenu des succès mitigés, plusieurs pays de la région ont mis en œuvre les recommandations de la résolution. Entre-temps, la République démocratique du Congo (RDC), l'un des pays les plus touchés, empêtré dans des conflits multiformes sans fin et une instabilité permanente, n'a pas pu mettre en œuvre un programme de lutte viable pour traiter le problème de la schistosomiase. Dans l'est de la RD Congo, pour la première fois depuis des décennies, on a exploré la morbidité, examiné les facteurs démographiques, géographiques et socio-économiques de 3 153 patients dans 57 villages situés sur une superficie de 65 658 km². La prévalence de la schistosomiase a atteint 90 % en certains villages, en particulier chez les enfants, ce qui correspond à des millions de cas non diagnostiqués et non traités dans cette seule région, s'étendant bien au-delà des points chauds historiques. Les facteurs prédisposant à la schistosomiase sont notamment la pauvreté, le manque de bonnes conditions sanitaires, l'accès limité à l'eau potable, les caractéristiques de la végétation, les activités en contact avec l'eau, le manque de connaissances sur la transmission et la prévention des maladies. Une morbidité importante a été constatée. Une pauvreté profonde a été mise en contraste avec la richesse des minéraux exploités ; la proximité des sites miniers n'a pas été associée à une réduction de la pauvreté ou à une diminution de la prévalence des maladies. Les infrastructures ont été gravement endommagées et la sécurité est fragile. Par le passé, les efforts nationaux en matière de santé ont laissé peu de traces, et les activités actuelles de confinement du virus Ebola à proximité ont éloigné le personnel des soins de santé de base.

En RDC, le fardeau des MTN en général, et celui de la schistosomiase en particulier, reste inconnu. Les enquêtes et les publications font cruellement défaut. La plupart des publications datent de l'époque coloniale. Pour la province de l'Ituri, la publication la plus récente sur la schistosomiase date de 1954. Malgré ces difficultés, la RDC a lancé un programme de lutte intégrée contre les MTN, y compris la schistosomiase, en 2012, mais la mise en œuvre n'a pas eu lieu avant 2016 dans la province de l'Ituri.

L'objectif principal de cette thèse est d'améliorer notre compréhension de la répartition géographique de la schistosomiase intestinale et des facteurs environnementaux, géographiques, socio-économiques et comportementaux qui sous-tendent sa transmission et sa propagation dans la province d'Ituri. Cette base de connaissances préconisera et facilitera la mise en place d'un programme efficace et durable de contrôle et de surveillance à grande échelle de la schistosomiase dans la province de l'Ituri.

Dans cette thèse, nous avons poursuivi cinq objectifs spécifiques. Premièrement, l'examen des outils de diagnostic actuels disponibles pour les milieux endémiques. Deuxièmement, présenter le contexte sous-jacent de la transmission et de la propagation de la schistosomiase intestinale dans la province de l'Ituri, en RDC. Troisièmement, évaluer la distribution de la prévalence et de l'intensité de l'infection à *S. mansoni* et déterminer les principaux facteurs de risque dans la province. Quatrièmement, évaluer la morbidité intestinale et hépatosplénique associée. Cinquièmement, décrire certains cas graves liés à l'infection par *S. mansoni*. À cette fin, trois enquêtes différentes ont été menées, à savoir (i) une enquête pilote auprès de 435 écoliers de 7 écoles primaires pour évaluer la situation ; (ii) une vaste enquête communautaire dans 51 villages de la province avec 2 131 participants d'un an et plus, hommes et femmes ; et (iii) une enquête approfondie et rigoureuse auprès des ménages dans 13 villages avec 1 022 participants, hommes et femmes d'un an et plus.

Les activités d'enquête sur le terrain liées à cette thèse ont donc été menées dans le cadre de trois campagnes de terrain dans la province de l'Ituri, à l'est de la République démocratique du Congo. Nous avons d'abord réalisé en 2015, une enquête pilote transversale impliquant 435 écoliers dans sept écoles primaires de trois districts sanitaires de la province, afin d'analyser la situation. Un seul échantillon de selles a été demandé et examiné selon la technique Kato-Katz (KK). Des données démographiques et anthropométriques (poids et taille) ont été recueillies et complétées dans un formulaire approprié.

Sur la base des résultats et des besoins identifiés en 2015, nous avons ensuite mené en 2016 une vaste enquête transversale systématique qui a couvert 2 131 participants de 51 villages de la province. Cette enquête visait à évaluer la prévalence et l'intensité de l'infection au sein des différentes catégories de la population âgée d'un an ou plus. Une fois de plus, le poids, la taille et les données démographiques ont été recueillis et un seul échantillon de selles a été demandé et obtenu par participant, et examiné selon la technique KK. En outre, une échographie abdominale a été réalisée sur un nombre limité de participants. Cette campagne de terrain a

permis de dresser une carte exhaustive de la prévalence de la schistosomiase, mais a également mis en évidence la nécessité d'améliorer les outils de diagnostic et de mieux comprendre la morbidité.

Pour répondre à ce besoin, en 2017, nous avons mené une enquête transversale rigoureuse et approfondie auprès de 1 022 individus issus de 145 ménages dans 13 villages choisis au hasard dans les zones où la prévalence s'est avérée relativement élevée lors des enquêtes précédentes. Les caractéristiques des ménages ont été recueillies. Les chefs de ménage ou leurs représentants ont répondu à un questionnaire approfondi sur les matériaux de construction de leurs maisons, le revenu du ménage, le bétail, les objets possédéd, l'accès à l'eau, l'assainissement, la présence et la qualité des latrines, l'utilisation des latrines. Chaque participant a répondu à un questionnaire individuel concernant l'état matrimonial, la profession, l'éducation, la religion, l'eau potable, le lavage des mains, l'hygiène corporelle, le nettoyage des vêtements, l'endroit où ils se lavent, où ils se baignent, l'agriculture, les activités de pêche, la possession de chaussures, les habitudes quant au port de chaussures, la consommation d'alcool, le tabagisme, la durée du séjour dans le village, la connaissance de la schistosomiase, sa transmission, sa prévention et son traitement, et ce qu'ils pensent de l'importance de la schistosomiase et, s'il s'agit d'un problème, les attitudes générales à son égard. Seuls les membres permanents du ménage ont participé. Les parents ou les chefs de famille ont répondu à quelques questions difficiles au nom de leurs enfants de moins de six ans. Ensuite, chaque participant a été pesé et sa taille mesurée. Il a été demandé aux participants de fournir un échantillon de selles par jour pendant cinq jours consécutifs. Le dernier jour, un échantillon d'urine a été demandé à 75% des participants qui avaient fourni au moins un échantillon de selles au cours de la période. Tous les échantillons de selles ont été examinés par la technique KK et l'urine a été testée par le test de l’antigène cathodique circulant (POC-CCA). Environ 85 % des participants ont subi des examens cliniques, notamment la palpation abdominale pour rechercher la possibilité d’un gros foie et d’une grosse rate, l'observation de la coloration de la conjonctive pour l'anémie, l'observation de la peau pour les éruptions et la gale éventuelles, et la percussion abdominale pour détecter l'ascite. La température corporelle a été mesurée chez les très rares participants qui ont déclaré avoir une perception actuelle de la fièvre. La plupart des participants qui ont subi des examens cliniques ont également subi des examens abdominaux par échographie pour estimer la taille du foie et de la rate, pour détecter à la fois l'hypertension portale et la fibrose, pour évaluer la taille, l'épaisseur et le contenu de la vésicule biliaire, pour détecter et classer les types d'ascite et pour rechercher toute autre anomalie des organes digestifs.

Tous les participants aux trois enquêtes successives ont reçu 500 mg de mébendazole pour le déparasitage général. Tous les individus trouvés positifs pour la schistosomiase par la technique KK ou le POC-CCA, seuls ou combinés, ont reçu 60 mg/kg de praziquantel pour le traitement de l'infection au schistosome détectée.

Dans la poursuite des cinq objectifs mentionnés ci-dessus, nous avons structuré nos résultats comme suit :

1 Nous avons fourni une mise à jour des réalisations actuelles pertinentes dans le domaine du diagnostic de la schistosomiase.

2 Nous avons documenté le contexte difficile de cette maladie, en particulier les guerres et les conflits armés en cours, la pauvreté et la ruée vers les minéraux précieux, impliquant également d'importants déplacements de population, qui ont largement favorisé la transmission et la propagation de la schistosomiase dans des zones auparavant non touchées par la schistosomiase en province d'Ituri, et qui ont empêché les efforts de détection ou de lutte contre la schistosomiase. Aujourd'hui, toute la province de l'Ituri est touchée par la propagation de la schistosomiase.

3 Nous avons décrit la répartition de l'infection à *S. mansoni* et son intensité parmi les catégories sociodémographiques et géographiques. La prévalence est élevée dans la province : dans certains villages, elle atteint 90 % ou plus des personnes testées, ce qui met en évidence la pertinence de l'existence d'une infection à *S. mansoni* non diagnostiquée dans un large segment de la population. La courbe de prévalence par âge et par sexe est typique des zones de forte endémicité où les programmes de lutte ne sont pas encore mis en œuvre. L'intensité de l'infection par *S. mansoni* est immense. Nous avons identifié que les principaux facteurs de risque de l'infection à *S. mansoni* étaient la pauvreté, le manque de latrines, d'installations sanitaires adéquates et d'eau potable, l'environnement immédiat qui pourrait favoriser la multiplication d'escargots d'eau douce hôtes intermédiaires, les activités en contact avec l'eau comme la pêche, le lavage des vêtements, la vaisselle, l'agriculture et également le manque de connaissances sur la transmission et la prévention de la schistosomiase. D'autres parasites intestinaux, notamment les ascaris, les ankylostomes, les trichocéphales, les ténia et même certains cas d'infection par *S. intercalatum* ont également été diagnostiqués.

4 Nous avons fourni, pour la première fois depuis l'époque coloniale, des données de base complètes montrant une charge de morbidité intestinale et hépatosplénique élevée dans la

province d'Ituri ; une charge qui est associée à l'infection par *S. mansoni* tant au niveau individuel que communautaire.

5 Nous avons signalé 8 cas graves dans quatre villages avec une prévalence très élevée de S. mansoni, allant jusqu'à 87,1%. Cinquante-six pour cent de la population présentaient une insuffisance pondérale dans les-dits villages. La morbidité intestinale et hépatosplénique était très fréquente.

En conclusion, la schistosomiase est un problème de santé majeur dans la province d'Ituri. La prévalence et l'intensité de l'infection, ainsi que la morbidité sont élevées. Plusieurs facteurs, dont la pauvreté, le manque de latrines, de bonnes conditions sanitaires, d'eau potable et d'éducation, favorisent la transmission et la propagation de la schistosomiase dans la province. De nouveaux outils de diagnostic sont nécessaires de toute urgence pour améliorer le diagnostic, la gestion des cas, la cartographie, l'élaboration de stratégies de contrôle et le suivi des programmes de lutte.

Ces conclusions appellent à des efforts concertés dans la mise en œuvre d'interventions de lutte efficaces susceptibles d'atteindre rapidement les segments les plus défavorisés de la population dans la province de l'Ituri. Ces interventions devraient inclure l'éducation de la population, l'amélioration de l'accès à l'eau potable, aux installations sanitaires et d'hygiène ainsi que la lutte contre les escargots. Les résultats de ces enquêtes peuvent contribuer à la planification de programmes de lutte efficaces et durables, basés sur des stratégies révisées et peuvent être utilisés par les décideurs de la province de l'Ituri, dans l'est de la République démocratique du Congo.

1 Introduction

Much effort has been undertaken to address Neglected Tropical Diseases (NTDs) control and elimination goals. After a long-lasting lobbying endeavour, NTDs have been included in the Sustainable Development Goals (SDGs) Agenda as it is stated in goal 3.3 « *By 2030 end the epidemics of AIDS, tuberculosis, malaria, and neglected tropical diseases and combat hepatitis, waterborne diseases, and other communicable diseases*» [1]. Such a commitment is a huge opportunity to be grasped. Mass drug administration (MDA) is the core aspect of these efforts and has brought several benefits in the way of NTDs control and elimination.

However, some challenges remain intact in the way of achieving of NTDs specific goals. Many countries, mainly in the sub-Saharan Africa (SSA) have not yet reached the World Health Organization (WHO) targets and/or timelines towards NTDs control and elimination. Therefore, it seemed urgent to ensure that «*country governments, decision-makers, and stakeholders of both public and private sectors, must join their efforts for mainstreaming and funding the control of NTDs ; scaling up NTDs programs to ensure no country, region, or community are left behind ; more advocacy from countries who do not reach WHO requirements and scaling up work in the countries with the greatest burden of NTDs ; completing NTDs mapping work that may provide accurate maps for decision-makers to identify communities in risk, for tackling an appropriate NTD, and to use more efficiently the available resources ; enhancing product development and operational research to support efforts to achieve NTDs targets and goals ; enhancing equity in the distribution of available resources and to reach the unreached* ». [2-5].

NTDs are numerous; they are both outcomes and drivers of poverty [1, 5, 6]. Goals, targets, and interventions toward control and elimination of NTDs are diverse. Each stakeholder must fully play its role. Joint endeavour is likely to bear much fruits. Together, the war against NTDs can be won.

Schistosomiasis, the third major NTD, remains a major public health issue in sub-Saharan Africa. In this region, the DRC is perhaps the country worst hit by all kind of epidemics in the world. In addition to schistosomiasis [7], there are thousands if not millions of cases of communicable diseases that daily plague the population. Although some are known, the problem lies in assessing their actual burden. As an indication, we quote – the list is not exhaustive – malaria, human African trypanosomiasis [8, 9], onchocerciasis [10], lymphatic

filariasis and loiasis [11, 12], soil-transmitted helminths, tapeworms, amebiasis, giardiasis, leprosy, cholera, typhoid fever, shigellosis, pneumonia and bronchopneumonia, meningitis, measles, poliomyelitis, yellow fever, monkeypox, Ebola, human immunodeficiency virus (VIH) and other sexually-transmitted infections, to name but a few [13]. Thus, one does not know where to put one's feet.

Since the World Health Assembly (WHA) resolution WHA 54.19 which stated, we quote: "endorsed as the best means of reducing mortality and morbidity and improving health and development in infected communities, the regular treatment of high-risk groups, particularly school-age children, and ensured access to single-dose drugs against schistosomiasis and soil-transmitted helminth infections in primary health care services, complemented by the simultaneous implementation of plans for basic sanitation and adequate supply of safe water" was launched in 2001 [14], special attention is paid to the control of schistosomiasis and great progress has been made. A number of control programs have been established to treat more than 75% of school-age children in the endemic areas. However, many targets were not attained. Since the London Declaration on NTDs and the WHA 65.21 resolution adopted in 2012 [15], which, we quote: "calls on all countries endemic for schistosomiasis: (1) to attach importance to prevention and control of schistosomiasis, to analyse and develop applicable plans with progressive targets, to intensify control interventions and to strengthen surveillance; (2) to take full advantage of non-health programmes to improve the environment, in order to cut the transmission of schistosomiasis and accelerate the elimination of the intermediate host; (3) to ensure the provision of essential medicines;", much progress has been made. However, much remains to be done.

The work presented in this thesis is focused on schistosomiasis and in order to understand some concepts relating both to NTDs and parasites, and their relationship with schistosomiasis, we will first discuss in the next paragraphs the NTDs problem, and introduce the basics of parasitic infections and then specifically on schistosomiasis.

We first carried out an exploratory study among schoolchildren to establish the existence of schistosomiasis in the Ituri province. Then, we did a large survey across the province to determine the prevalence of schistosomiasis among communities. Finally, we conducted an in-depth survey for assessing the burden of schistosomiasis in terms of prevalence, intensity, and morbidity. This last survey allowed us to simultaneously evaluate the reliability of current diagnostic tests used in the diagnosis of schistosomiasis.

1.1 Neglected tropical diseases (NTDs)

NTDs are a diverse group of communicable diseases that afflict more than one billion people from 149 countries worldwide [6]. They are so called because most of them occur in tropical and sub-tropical countries. They are caused by different pathogens including viruses, bacteria, protozoa, fungi, and helminths [16]. Approximately, 20 diseases are considered as NTDs and they share certain characteristics, including being a cause of immense pain, disability, and deaths ; affecting mostly people with low income and with limited access to basic health services, safe water, sanitation, and hygiene - poorest ; being chronic thus likely to sometimes cause irreversible damage ; and causing the stigmatization, rejection, and exclusion of the affected people by their communities [5, 17, 18].

NTDs are both outcomes and drivers of poverty [1, 19-21]. In fact, they result in not only poverty but also fuel poverty, and thus create a vicious circle of poverty-NTDs. Indeed, NTDs have a huge social and economic impact on the affected communities. These impacts mainly concern the loss of work and/or of the ability to be productive, the cessation of schooling, and the loss of self-image, which leads to psychologic disorders that are harmful to mental health, which further aggravates stigmatization [17, 22]. People living in sub-Saharan Africa (SSA), Brazil, Yemen, India, Bangladesh, and China bear the highest burden of NTDs estimated to be about 20.26 million disability-adjusted life years (DALY) [23].

The list of NTDs is summarised in Table 1.1. It appears clearly that the most debilitating NTDs are caused by helminths, protozoa, and bacteria [2-4].

Table 1.1: Neglected Tropical Diseases (NTDs) classification by cause and severity

Cause **Severity**	**Viral and other cause**	**Bacteria/Fungi**	**Parasites** **(protozoa and helminths)**
Debilitating	Dengue fever, chikungunya and rabies (viruses), Snakebite envenoming (animal)	Buruli ulcer and yaws (bacterial), Mycetoma, chromoblastomycosis and other deep mycoses (fungal)	Cysticercosis/taeniasis, echinococcosis, and food-borne trematodiases, and fascioliasis (helminths)
Most debilitating		Leprosy and trachoma (bacterial)	Chagas disease, human African trypanosomiasis, and visceral leishmaniasis (protozoa) Schistosomiasis, dracunculiasis, lymphatic filariasis, onchocerciasis, soil-transmitted helminths (helminths)

1.2 Parasitism and parasitic infections

Located at the heart of ecosystems, a human is an example of a particular ecosystem, a microcosm in which he greatly influences and at the same time is influenced by the ecosystems' effects. Liquid media, relative humidity, oxygen, pH, the presence of nutrient and the presence of inhibitory substances are all factors that can affect the development of living organisms. Parasites are living organisms unable to support themselves and parasitism is one of the categories of symbiotic associations in which one partner, the parasite, exploits the other partner, the host [24]. The host and the parasite are in permanent interactions. The parasite can colonize its host, settle in it despite the threats of the host immune system, and thus ensure its transmission to new hosts. The fact that the parasite exploits its host suggests that it is harmful to the host and so can cause a pathogenic effect (the disease) and even affect the life of the host. Pathogenicity is an essential concept for parasitism in which a parasite has a deleterious effect on its host. Parasites, which represent a little more than half of the existing species, are known for their potentially detrimental influence on human health and wellbeing [25]. They are likely to endanger entire populations, especially in the poorest parts of the world. To better understand these concepts relating to parasite and parasitism, as well as their application to schistosomiasis and the means of combating it, the aim of this section is to show some aspects of parasitism and the relationship the parasite has with its environment.

Parasites are diverse [26]. There is a variety of parasites ranging from viruses to metazoans, including prokaryotes. Strictly speaking, parasites are eukaryotic organisms (protozoans, helminths, and arthropods). Helminths and arthropods, for instance, are metazoans, therefore eukaryotic parasites. From the point of view of their intimacy with their hosts, one distinguishes

most often the ectoparasites, living on their host, and endoparasites which live in the body of the host and are likely to cause infections. Some parasites are obligatory parasites which present a strict need of an appropriate host to complete their life cycle, the host without which they could not exist. Parasites are metabolically and/or physiologically dependent on the host. Thus, they most often resort to a particular type of host which can be a definitive host or an intermediate host. A definitive host is one in which a parasite reaches sexual maturity, usually followed by sexual reproduction within that host, whereas in an intermediate host a parasite undergoes a required developmental step and may even reproduce asexually. The life cycle of the parasite can be direct (monoxenous), involving passage within a single host, or indirect (heteroxenous), which means that the parasite lives in several hosts during its life cycle. However, in some cases, the obligate parasite resorts to a particular host in order to reach a certain stage of essential development. Other parasites are simply optional and/or opportunistic. Some parasites are macro-parasites (helminths, for example), which are visible to the naked eye, in contrast to microparasites such as protozoans, which can be observed only under the microscope [27].

1.3 What is schistosomiasis?

Known as bilharziasis, with respect to the German physician Theodor Maximillian Bilharz who was the first to discover the parasite in 1851, schistosomiasis is a water-related parasitic infection caused by the digenetic trematode of the genus *Schistosoma*. The word '*schistosomiasis'*, named given by David Friedrich Weinland in 1858 by virtue of the typical morphology of the male parasite, is a contraction of two Greek words: '*schistos'* meaning '*split'* and '*soma'* that means '*body'*. Currently, 23 species have been formally recognized as belonging to this genus and some 6 to 8 species can infect humans and lead to morbid signs and symptoms. As an heteroxenous parasite, *Schistosoma* involves the interference of two hosts in its life cycle: a vertebrate and an invertebrate. *Schistosoma* species can infect several animals including both humans and other mammals [28-32].

Schistosomes are parasites of phylum Platyhelminthes, class of Trematodes and family *Schistosomatidae* that includes digenetic trematodes [25, 33, 34]. Human schistosomiasis is caused by six species of the genus *Schistosoma* summarised in Table 1.2 [35, 36]. Apart from the six above-mentioned, there are two new coming species also pathogenic to humans: *S. matthei* (Southern Africa) and *S. malayensis* (Malaysia) [28, 32, 37]. However, the first four species cause the most burden. Schistosomiasis is the third most devastating NTD and one of

the major poverty-related diseases [14-16]. According to recent estimates, nearly 800 million people are at risk, and 221 million of whom are infected in 78 countries, causing about 70 million disability-adjusted life years (DALY) and high morbidity in Africa, Brazil, the Caribbean, the Middle East, and South-East Asia. More than 92% of infected people live in sub-Saharan Africa [38-40]. As mentioned above, the WHA 54.19 resolution recommended to governments in endemic countries to employ the preventive chemotherapy (PCT) strategy for reducing morbidity due to schistosomiasis [14, 38]. However, it was reported that after ten years the target coverage was not attained. Then the WHA resolution 65.21 set new objectives and urged governments of endemic countries to intensify schistosomiasis control programs [15]. Likewise, in 2014 the global coverage was less than 21%, which cannot help stop transmission of schistosomiasis [38, 40].

Table 1.2: Status of schistosomiasis in endemic countries, by continents: 2017

Continents	**Number of estimated cases**
Africa	199,612,090
Americas	1,623,107
Eastern Mediterranean	16,652,623
South-East Asia	21,327
Global	220,742,768

Source: [41]

Table 1.3: Types of schistosomiasis, *Schistosoma* species, intermediate snail hosts, people at risk, and distribution

Types	***Schistosoma* species**	**Intermediate snail hosts**	**People at risk (million)**	**Distribution**
Intestinal	*S. mansoni*	*Biomphalaria* (*Planorbis*) spp	393*	Africa, Middle East, Caribbean Islands, Brasil, Venezuela, Bolivia, Suriname
	S. japonicum	*Oncomelania* spp	40**	China, Indonesia, the Philippines
	S. mekongi	*Neotricula* spp	0.06	Cambodia, Lao People's Democratic Republic
	S. guineensis and *S. intercalatum*	*Bulinus* spp	Unknown	Rainforest areas of Central Africa
Urogenital	*S. haematobium*	*Bulinus* spp	436	Africa, Middle East

Sources: [35, 42]

The discovery of aquatic snails as intermediate host by Robert Thomson Leiper in 1902 was the decisive turning point of understanding the complete life cycle of *Schistosoma* spp [37]. Similar to other trematodes, *Schistosoma* species present complex life cycles including both free-living and parasitic forms (Figure 1.1). The main species of fresh-water snails intermediate hosts found in Africa are summarised in Table 1.4.

Table 1.4: Main species of fresh-water snails intermediate host found in Africa

Family	**Sub-family**	**Genera**	**Species**
Planorbidae	*Planorninae*	*Biomphalaria* (*Planorbis*)	*Biomphalaria glabrata*, *B. pfeifferi*, *B. tchadiensis*, *B. camerunensis*, *B. alexandrina*, *B. sudanica*, and *B. chaonomphala*
	Bulininae	*Bulinus*	*Bulinus globosus*, *B. truncatus*, *B. umbilicatus*, *B. jousseaumei*, *B. camerunensis*, *B. forskalii*, *B. guernei*, and *B. obtusus*

Sources: [43]

People become infected from contact with water. In the water, infective stage of the parasite, the cercariae, swim and penetrate through the epidermis of human host, shedding their forked tails, and becoming schistosomulae. The schistosomulae migrate into the host's circulatory system, then migrate to the lungs, the heart, and finally to the liver, where they feed on blood and develop to become either female or male adult worms, within 6 to 8 weeks after exposure. These female and male adult schistosomes pair, copulate and then exit the liver via the portal vein system to begin reproducing. Female (measuring 7-28 mm depending on species) and male

adult worms may live up to 30 years, either in the inferior mesenteric veins draining the large intestine (*S. mansoni*), the superior mesenteric veins draining the small intestines (*S. japonicum*), the lower section of the inferior mesenteric plexus and the rectal venules (*S. interacalum*, and *S. guineensis*), or the vesicular and pelvic venous plexus of the bladder (*S. haematobium*). However, they are capable of moving between these sites. Fertilized females deposit about 300 (African species) to 3 000 (Asian species) eggs daily in the small venules of the portal and peri-vesical systems. The eggs are moved through the surrounding tissues progressively toward the intestine lumen and are released into the environment through stools (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis*) or toward the lumen of bladder and the ureters and are then eliminated through urine (*S. haematobium*). Schistosome eggs that reach freshwater, under certain conditions, hatch and release a larva, the miracidium, that swims by ciliary movement to locate and penetrate specific and suitable snail intermediate host. In the snail, miracidium develops into mother and daughter sporocyst stages respectively, and finally generate thousands of cercariae stages. Cercariae are liberated from the snail into freshwater where they may survive about 12 to 72 hours looking for the specific mammalian definitive host [27, 44].

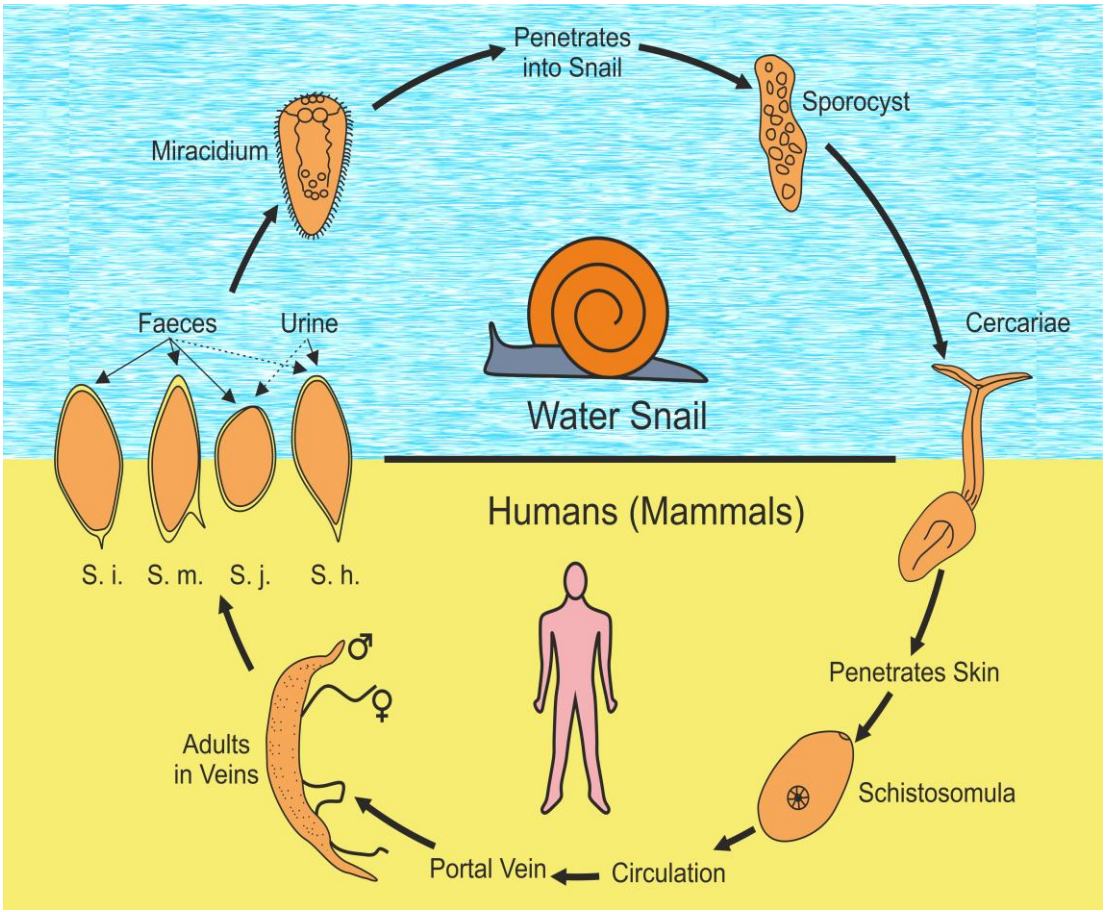


Figure 1.1: *Schistosoma mansoni* life cycle (1) Paired adult worms (female is thin, male is bigger), (2) Eggs (see note below), (3) Ciliated *miracidium*, (4) *Biomphalaria* intermediate host snail, (5) Sporocyst, (6) *Cercariae*, the infective stage, and (7) Schistosomula, the young parasite.

Legend: S. i.: *S. intercalatum,* S. m.: *S. mansoni,* S. j.: *S. japonicum,* S.h.: *S. haematobium*

Pathology in schistosomiasis may progress from early to chronic and advanced stages [25, 44-48]. Depending on the species, early manifestations may either be a banal rash (all the species), a maculopapular eruption resulting from the percutaneous penetration of schistosome cercariae, to a more serious 'swimmer itch', an immune reaction among sensitized people who were previously infected and who become re-infected by non-human schistosomes, or an acute and serious Katayama syndrome (*S. japonicum*), an acute and systematic manifestation which occurs several weeks after infection. People with Katayama syndrome may have signs and symptoms such as fever, headache, body aches, or myalgias, fatigue, malaise, non-productive cough, and bloody or non-bloody diarrhoea [45, 49, 50]. Many people do not manifest symptoms at the early stage. In later stages, schistosome infections are frequently associated

with chronic inflammatory response to antigens of schistosome eggs trapped in the tissues which provoke complex granuloma formation. These later manifestations depend on the species of parasite. Granuloma formation is a CD4+T-helper-2 mediated response. It begins with accumulation of defence cells such as macrophages, neutrophils, and eosinophils around the newly entrapped egg. As the granuloma matures, epithelial precursor cells, fibrocytes, and subsequent plasma cells and other lymphocytes form a peripheral layer around the lesion. During this stage the eggs may be destroyed. Ultimately, fibrocytes, collagen and other extracellular components become the dominant feature of the granuloma [25]. Granulomas may lead to host tissue fibrosis and may facilitate the passage of schistosome eggs to intestine or urinary tract lumen [45].

Intestinal schistosomiasis, caused by *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. intercalatum*/ *S. guineensis* is due to schistosome eggs retained in the intestine wall, which through inflammatory response, set off inflammation, hyperplasia, ulceration, micro-abcess, and polyposis. The resulting granulomatous lesions may cause abdominal pain, diarrhoea or constipation, intestinal polyps' and ulcers' bleeding, with haematochezia [44]. In chronic [51] and long-lasting infections with *S. mansoni* and *S. japonicum*, granulomatous inflammatory responses to eggs embolized in the liver inducing presinusoidal inflammation with hepatomegaly and periportal fibrosis. Obstruction of the blood flow consecutive to collagen deposits may lead to portal hypertension, with high blood pressure around the digestive organs, ascites, and ultimately varices formation whose rupture can lead to variceal bleeding with hematemesis and/or melaena. Splenomegaly and hypersplenism are frequently associated with intestinal schistosomiasis. However, it is important to make the causal diagnosis in order to rule out splenomegaly caused by to malaria in endemic areas [52, 53].

In urinary tract schistosomiasis, caused by *S. haematobium*, granulomatous inflammatory response to deposited eggs lead to dysuria and haematuria, the first manifestations of the disease which appear 10 to 12 weeks post-infection. Chronic stages' symptoms may include anaemia, nephrotic syndrome with proteinuria, bladder calcification, and ureteric obstruction. Secondary bacterial infections of the urinary tract, renal colic, hydronephrosis, and renal failure may occur. Urinary tract schistosomiasis chronic lesions are frequently associated with bladder cancer and genital organs' lesions which may also facilitate the transmission of human immunodeficiency virus (HIV) [54]. Both intestinal and urinary tracts' schistosomiasis characteristic lesions may be visualized with ultrasound examination [55].

In rare cases, infections with *S. mansoni*, *S. haematobium*, and *S. japonicum* can affect the central nervous system and be at the origin of neurological complications well known as neuro-schistosomiasis [44, 56-58]. These complications due to egg deposition following aberrant migration of the adult parasite to the spinal cord and brain may occur early in the course of infection. They result from the mass effect caused by both the multitude of eggs and the large spinal and cerebral granulomas that are formed. Signs and symptoms of neuro-schistosomiasis include increased intracranial pressure, myelopathy, and radiculopathy. The complications of cerebral neuro-schistosomiasis include encephalopathy with headache, visual disturbances, delirium, seizures, and motor deficit with ataxia. While spinal complications include lumbosacral pain, lower limb radicular pain, muscle weakness, sensory loss, and bladder disfunction [44, 45, 51].

Children bear the highest toll of schistosomiasis [59-64]. They are at the same time the most affected and the main transmitters of the disease in the community. Indeed, their love of playing in the water and the elimination of their excreta in the environment contribute greatly to the spread of the disease as well as being the main victims in the endemic areas. Several population studies conducted among children have shown that schistosomiasis is often the cause of stunted growth, anaemia, and cognitive and memory disorders that limit their potential, particularly in terms of school performance and social integration. However, these disorders can be corrected, at least partially, by a good control program that specifically target this vulnerable category of the population [40, 65, 66]. Note that schistosomiasis may also affect the health of the mother and the unborn foetus [44, 67] mainly through the indirect effects of maternal anaemia and/or immune disorders.

Other manifestations of schistosomiasis are pulmonary and renal diseases [44, 45].

Diagnosis of schistosomiasis is based on both a complete and targeted patient history, the use of self-administered questionnaires, clinical, laboratory, and medical imaging examinations [44, 45, 68-70]. Some key indicators may show a possible positive diagnosis of schistosomiasis. They include [44, 52] anamnesis which must include patient stay or provenance from an endemic region, possible contact with surface water likely to be infested by freshwater snails (lakes, rivers, streams, ponds or any other permanent water cluster). Physical examination must note the observation of any rash (maculopapular or erythematous lesions) that may result from penetration of the cercariae through the skin. Palpations of the abdomen and measurements of the liver may reveal hepatomegaly when performed, described, and graded following the

protocol [52, 71-73]. Splenomegaly may be detectable by measuring the extension of the spleen below the rib cage when measured in the left MCL and the left mid axillary line (MAL). Spleen consistency must also be described and graded. Finally, the search for possible generalized lymphadenopathies might be conducted [52, 72-74].

Laboratory investigations may be based on parasitological, immuno-serological, and molecular methods. Current laboratory tests for schistosomiasis diagnosis are mainly based on direct parasitological detection, detection of circulating antigens and/or specific antibodies, and on molecular methods. More detailed information can be found in focused literature [75, 76]. As the background of evaluation for emerging and future test modalities, the current laboratory available tests are summarized in the next paragraphs.

The reference method for laboratory diagnosis of schistosomiasis is parasite egg search in the infected individual's stool, urine, or tissues [48, 77]. These methods are widely used due to their relative efficacy and moderate cost effectiveness for case-management, screening or surveillance. However, they have some drawbacks such as decrease of sensitivity when there is no egg excretion, principally in areas of low endemicity [78]. For intestinal schistosomiasis, Kato-Katz (KK) [79] remains the gold standard technique, even if other techniques exist [80-91]. The KK technique has the advantage of egg quantification in fresh stool samples yielding infection density [92]. For urinary schistosomiasis, direct microscopy of filtered urine is the reference method [93].

Antigen detection (AgD) assays are promising diagnostic tools, are non-invasive and permit large-scale sample testing [94, 95]. However, they are frequently false negative [96] and often are not species specific [97]. AgD assays need to be evaluated further. However, they are not currently considered as suitable replacement for traditional diagnostic tests [98, 99].

Diagnosis of *Schistosoma* may also resort to antibodies detection (AbD) [100]. Most AbD assays measure serum immune reactivity to schistosome antigens. AbD assays exhibit a modest sensitivity and a limited specificity although several antigens are available for diagnostic purposes [101]. They are more attractive for monitoring areas of controlled transmission and are important for the diagnosis of atypical forms of schistosomiasis [57, 102] and among travellers without egg excretion and for imported schistosomiasis [103]

Detection of haematuria and proteinuria are other alternative ways used for indirect diagnosis and screening tool for schistosomiasis [104, 105]. Haematuria and proteinuria are mostly

associated with *S. haematobium* infection. They are relatively cheaper and more specific. However, their sensitivity is variable [106].

To overcome the shortcomings of both parasitological and immunological methods, development of more sensitive and more specific molecular diagnostic tools diagnosis opened new perspectives. DNA detection techniques may evolve into potentially valuable tools in schistosomiasis diagnosis. DNA-based assays have also proven to be useful for cure assessment [107] in a variety of sample types comprising blood, urine, faeces and saliva by polymerase chain reaction PCR [108] or by the newly developed loop mediated isothermal amplification (LAMP) [109] which are highly sensitive and specific and have improved schistosomiasis diagnosis [110-112]. However, they require the use of highly skilled personnel and expensive equipment.

At present, the first choice for the treatment of schistosomiasis is now the use of praziquantel, an acylated quinolone-pyrazine molecule, which is effective in 70-100% cessation of egg production after a single oral dose. It is low cost and has a wide therapeutic spectrum on the three most prevalent species: *S. mansoni*, S. haematobium, and S. japonicum. Even if its anthelmintic action remains uncertain, many think that it inhibits the sodium-potassium ATPase (Na+-K+) of the adults worms by increasing the membrane permeability to certain monovalent and divalent cations such as calcium (Ca2+), and leading to spastic paralysis [113]. Praziquantel is only effective on adult worms, but not on juvenile nor egg stages because it is rapidly metabolized after oral administration and is converted to inactive compound. For this reason, Borrego-Sanchez et al. proposed the use of molecular and crystal structure of praziquantel [114], whereas Frezza et al. the liposomal-praziquantel [115], El-Moslemany et al. other nanoparticles such as miltefosine lipid nano-capsules [116], and Tomiotto-Pellissier et al. solid lipid nanoparticles, nano-emulsions, and polymeric nanoparticles [113] in order to increase the effectiveness of the products. Other complementary drugs comprise Oxamniquine and Artemisinine.

As soon as the causal parasite was identified and its life cycle determined, the fight against schistosomiasis began. It first consisted of trial and error. However, the experiences gained over the years permitted several countries around the world to fight successfully against schistosomiasis. This success resulted from substantial human, material, technical, and financial investments. Indeed, like any living organism, the parasite has survival instincts including successful reproduction and propagation. It must therefore be hunted everywhere and

with all existing and new means of control. In the absence of an effective vaccine, control measures have always been based only on some groups of strategies: strategies for reducing transmission, such as education of population, water, sanitation, and hygiene measures, and chemotherapy against the parasite [25]. In the meantime, the strategy of reducing transmission by snail control, improved water supply and sanitation. Snail control by manual capture as early recommended by Katsurada [31] quickly showed its limits. Then, chemical molluscicides use became mandatory and successful, except their collateral effects on other organisms. Although expensive, improved water supply and sanitation, environment management, and education proved to be potential means of control of schistosomiasis. In this way, some countries have proved that it is possible to effectively control, stop transmission, and eliminate schistosomiasis. Here, we will cite only two examples: Japan and China.

Japan remains the best example of success in the fight against schistosomiasis. After the discovery of S. japonicum in 1904 and that of *Oncomelania* intermediate host snail in 1913, a merciless fight was waged against schistosomiasis. Soon control efforts received the unwavering support from all categories of population. The inhabitants of the affected villages, the sick persons, various organizations, and both regional and national government showed their commitment. Taken as a whole, the fight against schistosomiasis lasted more than a hundred years. Started at the initiative of a village leader, it rapidly received the collaboration of the government. Then a wrestling association was created which was quickly reinforced by several other local associations. The main association began its activities in 1950 and schistosomiasis was declared eradicated in Japan in 1996. The control strategy was based both on snail control and chemotherapy, including the use of praziquantel. However, the key of its success resulted from multi-sectoral contributions including education of the population, improvement of water supply and sanitation. As remainder, in 2012, the coverage of these two important services was 100% in the country. As a logical consequence, the average life expectancy grew from 47.8 years in 1947 to 80.55 years in 2011, a jump of more than 32.7 years [31].

China’s efforts in the fight against schistosomiasis became earnest when its importance for public health and economy was recognized by Mao Zedong in 1950. Since then, the political will coupled with substantial technical and financial supports have made it possible to an integrated approach to fight. This allowed a drastic reduction in the burden of the disease. In fact, from more than 11 million cases in the 1950s, schistosomiasis prevalence was reduced to less than 1 million cases in 2004. From this time, a new integrated strategy was set up. It was

based on improved environmental management, the source of infection, chemotherapy, snail control, health education, and improvement of sanitation and of access to safe drinking water. In 2015, estimates showed that this new strategy had reduced the prevalence by 90.8%. Indeed, data from that year showed that there were 77.2 thousand cases throughout China. A real giant step. This opens a royal road for the interruption of schistosomiasis transmission in 2020, its elimination in 2025, leading to the acceleration of economic development of the formerly affected regions [117]. Approaches, strategies and tools of control used by China could be advantageously transposed in Africa, the continent hardest hit by schistosomiasis, if there was the same commitment.

Biological control has been advocated for many years. It is based on the introduction of predatory such as molluscivorous crabs and fish. Crab species such as *Potamon didieri* and *Potamon lirrangese* were successfully experimented alongside with *Xenopus laevis victorianus*, *Serranochromis macrocephala* and *Chrysichtys mabusi* fish species. *Chrysichtys mabusi* fish was found to be strictly mollusciphage [118]. Recently, some authors proposed prawn aquaculture for both poverty alleviation and schistosomiasis control [119]. Such initiatives are to be promoted and expanded.

Development of schistosomiasis vaccine candidates is ongoing. Some candidates such as Bilhvax (against *S. haematobium*), and *Sm*-TSP-2 and *Sm*-14 (against *S. mansoni*) are at proof of concept (POC) clinical trials [120] and other in search [121].

1.4 Schistosomiasis in DRC

In DRC, the first cases of schistosomiasis were detected and mentioned by Firket while the Universal Exhibition of Brussels took place in 1897. Since then, it was well documented by the colonizer until 1960. Consequently, between 1911 and 1926, many endemic areas were located in Kinshasa (the capital city), Lubumbashi at the southeastern side, in the Uele region and the western region of the country. Three species of the genus *Schistosoma*: *S. mansoni*, *S. haematobium*, and *S. intercalatum* were reported. Few cases of human infection by *S. rhodaini* were also reported. The first comprehensive study of the distribution of schistosomiasis in DRC was done by Gillet and Wolfs in 1954 [118]. The distribution of *S. mansoni* is shown in Figure 1.2. Since then, several surveys were carried out and the distribution of schistosomiasis in DRC updated. *S. mansoni* was reported in four geographical regions of DRC: Kinshasa and regions around the western side, the Uele regions in the northern side, Kibali-Ituri and Kivu regions in the eastern side, and Katanga and Kasaï regions in the southern side of the country. In the first

area, cases of *S. mansoni* were reported in Kinshasa and as far as the Atlantic Ocean coast. In the Uele regions, high prevalence between 70.0-93.0% of schistosomiasis was reported among rural populations at various locations. In the eastern regions, the foci of the disease were reported along the lakes Albert, Edward, Kivu, and Tanganyika. On the shore of the lake Albert the prevalence varied between 60.0-70.0%. Around the mining centers, it varied between 40.0-51.0%, whereas it was low between 6.5-20.0% in the southern side of Ituri. Note that the first cases of schistosomiasis were reported by Scops in 1926 in the Ituri province. The prevalence was low between 0.8-32.0 in the Kivu region. For the southern regions, prevalence varied greatly between 10.0-100% from one location to another depending on the proximity to lakes and rivers, mining activities, and rural or urbans populations [122]. After the independence, very few publications on schistosomiasis were produced in DRC [53, 62, 123-128]. Recent estimates show that there are 15 million of schistosomiasis cases in DRC [7, 129], whereas others consider it unknown [13].

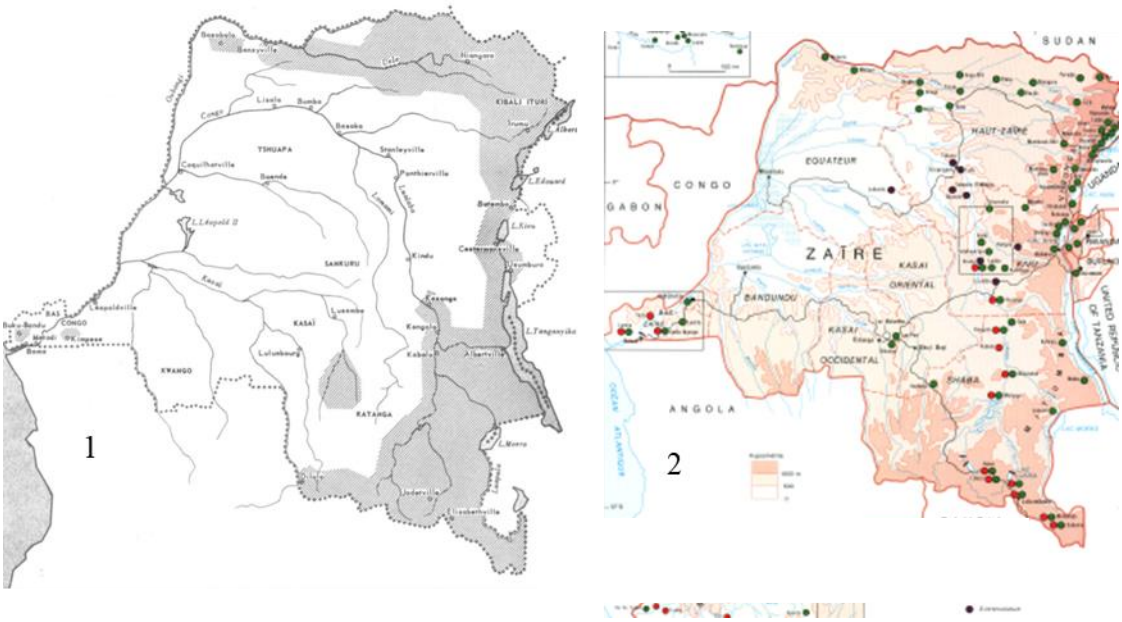


Figure 1.2: Historic distribution of schistosomiasis in Congo. Adaptated from: 1) *S. mansoni* in 1954, By J. Gillet and J. Wolfs. [118] and in 2) *S. mansoni* (green colour dots) and *S. haematobium* (red colour dots) in 1987, By Doumenge et al.[122].

1.5 Purpose

The purpose of this section is to highlight the global commitment to eliminate Neglected Tropical Diseases (NTDs) and to explain why we undertook this study. We will focus on the case of schistosomiasis, one of NTDs for which there is a call for elimination. Since the adoption of resolution WHA 54.19 of the World Health Assembly in 2001 [14], a resolute and continuous war has been waged against schistosomiasis, a disease which, together with other NTDs, affect more than one billion of people around the world. A race against time is engaged to eradicate this global scourge [18, 130]. We must seize this opportunity because, on the one hand, these diseases are now receiving sustained and unprecedented attention and, on the other hand, the proposed control programs are using strategies that are very cost-effective [40]. Since 2003, several countries in sub-Saharan Africa, the region with the highest incidence of the disease, have committed to launch and implement their national control programs. These countries include Uganda, Niger, Burkina-Faso, Kenya, and Ethiopia. Democratic Republic of Congo (DRC), the second largest country in Africa, is one of most affected nations. Indeed, entangled in endless conflicts and chronic instability, the DRC remained focused on itself. Moreover, in DRC the real burden of NTDs in general and that of schistosomiasis in particular are unknown [13]. The World Health Organization (WHO) [40, 65, 66] states that schistosomiasis mapping is particularly relevant prerequisite for good planning of control programs. Indeed, schistosomiasis is endemic in the DRC. It was discovered there around 1897 and was regularly documented by the Belgian colonizer [118]. Thus, the most detailed review on the disease is dating from 1954 [123]. Since the independence of DRC at 1960, few papers have been published on schistosomiasis, most of them concern only few counties in the eastern, central, and western sides of the country [124, 125]. Publications that are less than twenty years old are even rarer and do not cover the national territory [53, 62, 68, 123, 126-128]. These existing publications are therefore not sufficient, given both the size and the variability of the country. The province of Ituri, in the extreme north-east of the DRC (Figures 1.3 to 1.5), is one of the regions where schistosomiasis was the best documented in colonial times [118]. However, since the independence of the DRC, only one publication was made on the disease in 1986 [131]. Although the control program finally started in 2012 in the DRC, no publication proves that a prior mapping of the disease was done. In 2013, a national health survey was conducted nationwide. Thus, the lack of reliable information on schistosomiasis suggests that the implementation of the control program may be based on questionable assumptions. However, the publication of the results of this survey is still awaited [132-134]. Many believe

that it is possible to extrapolate data on schistosomiasis from other neighbouring countries and regions that have the same characteristics or from reviews or compilation of data collected from health care services [135-137]. Yet systematic reviews often have their own limitations and do not allow a description of updated information on a disease [130]. Targeted survey data from a representative sample of the country or region are the only ones likely to provide the necessary information to enable good mapping for adequate planning of control programs. The control program initiated in 2012 was launched in the Ituri province only in 2016. The strategy used, aimed at treating children aged 5 to 14 once a year, is based on the criterion of prevalence varying between 10.0 and 49.0%. Yet, the informal data we obtained, resulting from several field surveys, show that the magnitude of the problem is much greater than one thinks. In the following paragraphs, we will first explain our motivation to carry out this study, then we will briefly describe the highlights of this thesis, and finally, we will shortly mention the aim, goals and objectives of this thesis.

1.6 Motivation

Entangled in endless conflicts and chronic instability, the DRC has almost remained on the side-lines of schistosomiasis control and elimination. Probably one of the most affected nations by schistosomiasis in the world, if not the first, the DRC began its control program in 2012, and for good reason. Firstly, mapping of the disease has not been done, making any serious planning impossible. Most of data used for planning the program come from health care services and therefore inappropriate as the existing mapping of schistosomiasis in the DRC goes back to colonial times. At that time, the disease was intensively investigated throughout the national territory and well documented. Unfortunately, since the independence of the country in 1960, few publications are done on schistosomiasis and the most relevant are more than 20 years old and concern only a few scattered sites, especially in the west of the country. For the province of Ituri, for instance, one of which schistosomiasis was best documented and mapped in the colonial era, the last reliable publication was done in 1954 and schistosomiasis was recognized as hyper-endemic in many parts of the province. At present, in Ituri most health services are ruined or non-existent. The control program only started in 2016 under the auspices of the national Ministry of Health and few months after the end of our large survey. The treatment strategy used focuses only on children aged 5 to 14 which means that schistosomiasis prevalence is below 50% in the province. Alarmed by our informal observations, based on our own results, we studied schistosomiasis burden nowadays in the Ituri province – including epidemiological data as well as prevalence, intensity, morbidity, and the main factors that may have contributed to the transmission and spread of schistosomiasis – in order to correct this underestimation and to take advantage of the extended support of the global health community. We believe that this approach would:

1. Help improving early detection of schistosome infection
2. Allow people who need the treatment to be treated
3. Get people involved in solving their health problems

1.7 Contribution of this thesis

Our thesis adds novelty in estimating the current burden of schistosomiasis for the first time in the Ituri province since colonial time. Indeed, it plays the role of pioneer on this ground after the independence of DRC. As a scout, it is clearing the path to build a sustainable and successful control program. This thesis states that, even schistosomiasis is endemic in sub-Saharan Africa, the situation may be radically different from one country to another and from one geographical area to another within the same country. Also, within the same location, many factors may contribute to the transmission of the disease between people. Therefore, this thesis argues that the real needs of the population of a region would be poorly understood if control programs are based on mere estimates.

In addition, our thesis demonstrates that the burden of schistosomiasis in the Ituri province is far greater than that known during colonization and even current data from health services. It commits itself to recommending these results as a starting point for thinking in depth about the health needs of the population. Above all, this thesis fills the glaring lack of reliable and recent data on schistosomiasis in Ituri and will guide decision-makers and stakeholders to exploit contained information in the best way and for the benefit of those in need. Incorporating these new uncovered data on schistosomiasis, this thesis aims to revise our understanding of the scourge of schistosomiasis in the DRC and has the advantage of shedding new light on the prerequisites necessary in the fight against schistosomiasis. The WHO through its resolution WHA 65.12 [15] has committed to the elimination of schistosomiasis. An illustration that schistosomiasis is not a fatality and that victory against it is possible is given by Japan, which succeeded in eradicating it in 2002 [31]. We focus on the Japan case as it shows that when people in a country or region decide together to overcome a deadly problem, they can. Since more than 40 years, several countries including Brazil , Morocco, Egypt, China, Cambodia, Mauritius, Oman, Jordan, Saudi Arabia, and Tunisia has successfully implemented control programs [35] and some of them are on their way to eliminate and eradicate schistosomiasis [138-140]. In sub-Saharan Africa few countries including Burkina Faso, Ghana, Niger, Sierra Leone and Tanzania have scaled-up schistosomiasis treatment to the national [35]. DRC is among those countries who follow suit and schistosomiasis has become a serious problem in the DRC and in the Ituri province.

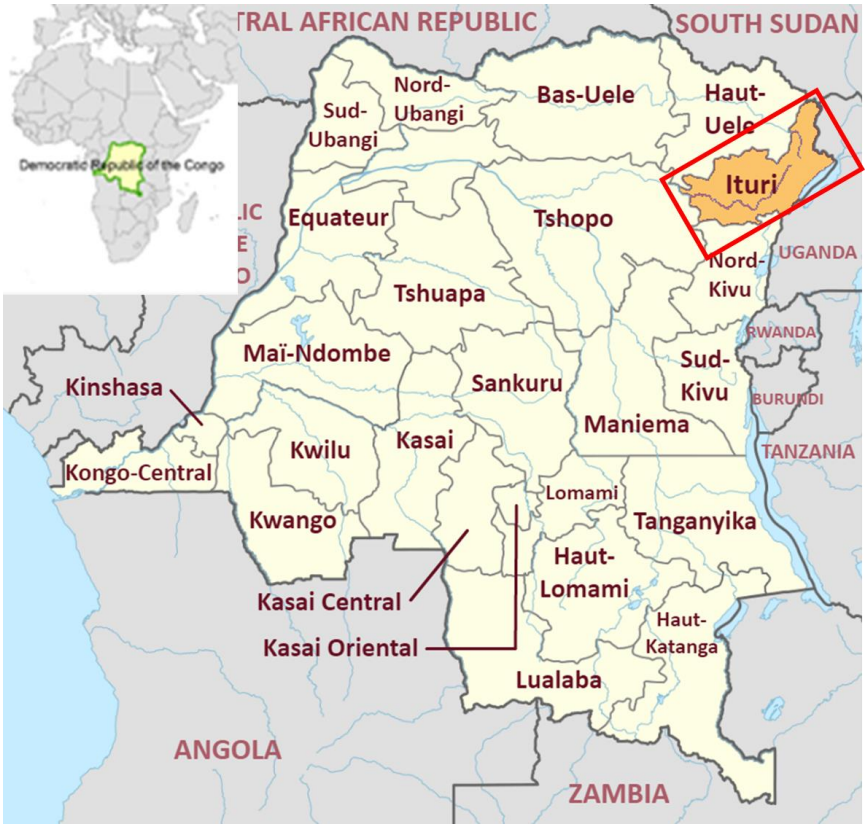


Figure 1.3a: Study site: Ituri, one of the 26 provinces of DR Congo.

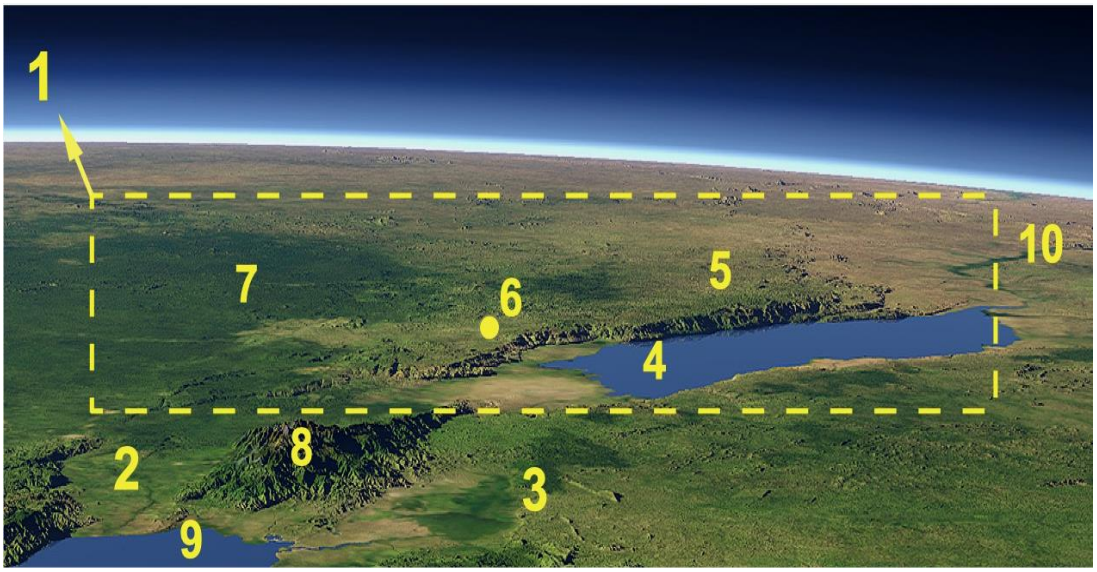


Figure 1.3b: Study site: 1) Ituri Province, 2) DR Congo, 3) Uganda, 4) Lake Albert – 615m altitude 5) Blue Mountains : Aburo – 2,445m altitude, 6) Bunia city, 7) Equatorial Forest (550m altitude), 8) Rwenzori Mountains – 5,110m altitude, 9) Lake Edward, and 10) Albert Nile. Adapted from Google Earth, Google Landsat/Copernicus. https://earth.google.com/web/@0.95431441,30.54511868,1290.44726964a,621662.89191239d,35y,30.19560411h,0t,0r .

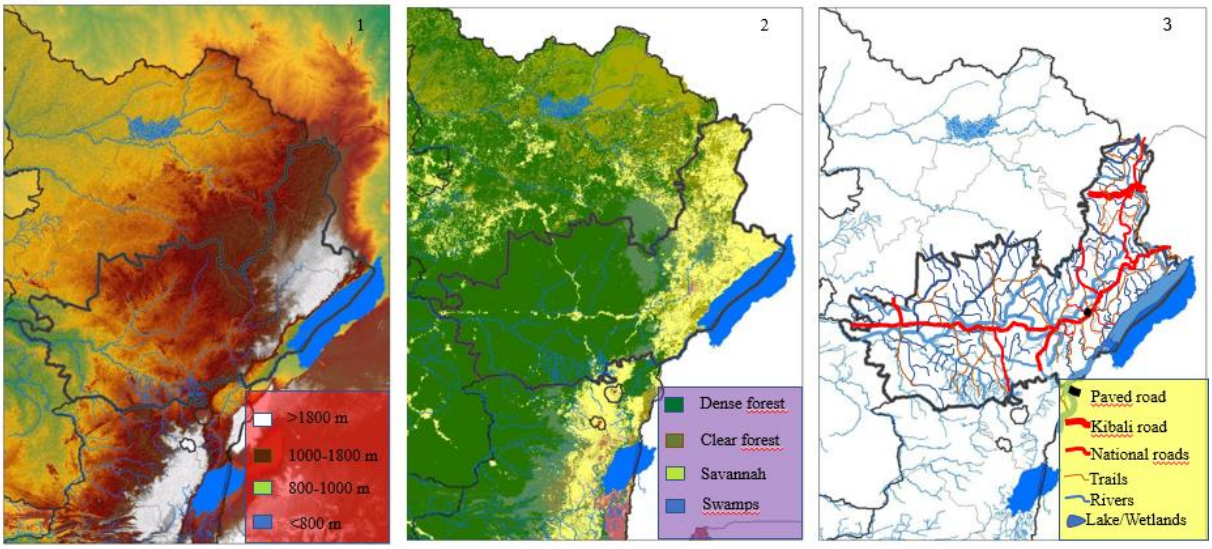


Figure 1.4: Study site 1) Altitude 2) Vegetation 3) Water distribution and roads

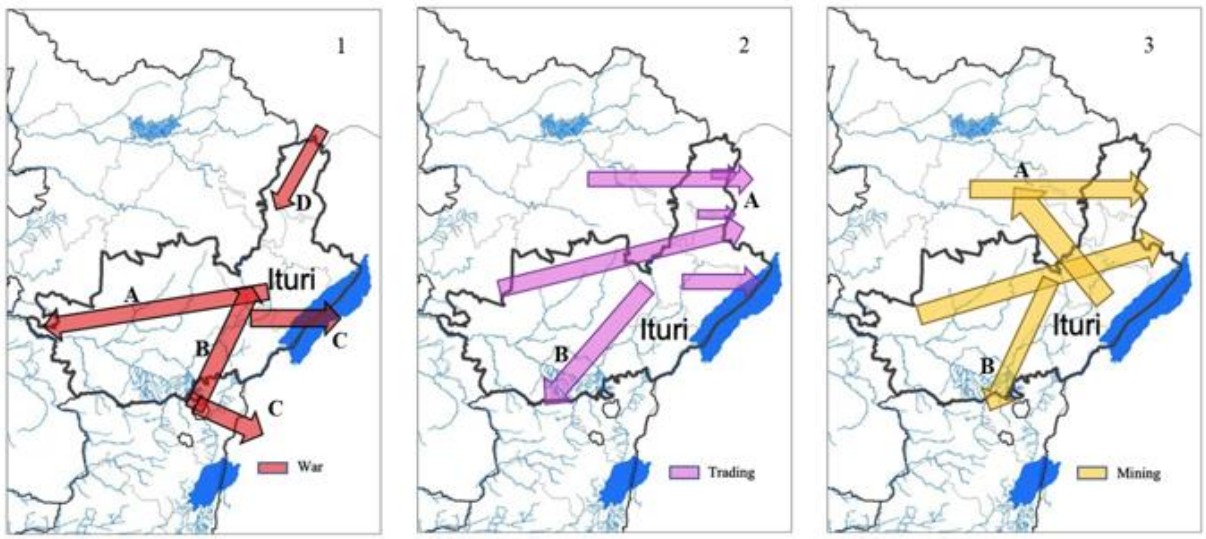


Figure 1.5: Study site: reasons of population movements: 1) *War*: (A) Several tens of thousands of Iturians fled to Kisangani or other western areas of DRC; (B) several tens of thousands of people from North-Kivu province came to Ituri for their security both because of rebellions and Ebola outbreak; (C) since 2017, more than 100,000 people left Ituri for their safety, reaching Uganda across Lake Albert or by other ways; Because of war, several tens of thousands of people from South-Sudan (D) came to Ituri for their security. 2) *Trading*: (A) The trade in the Ituri province for both import and export is essentially oriented towards neighboring Uganda, but sometimes to North-Kivu province (B). 3) *Mining*: Big gold mines, such as Kibali Gold Mine (A) attract gold trade. Much of the gold from artisanal mining is sold in Uganda.

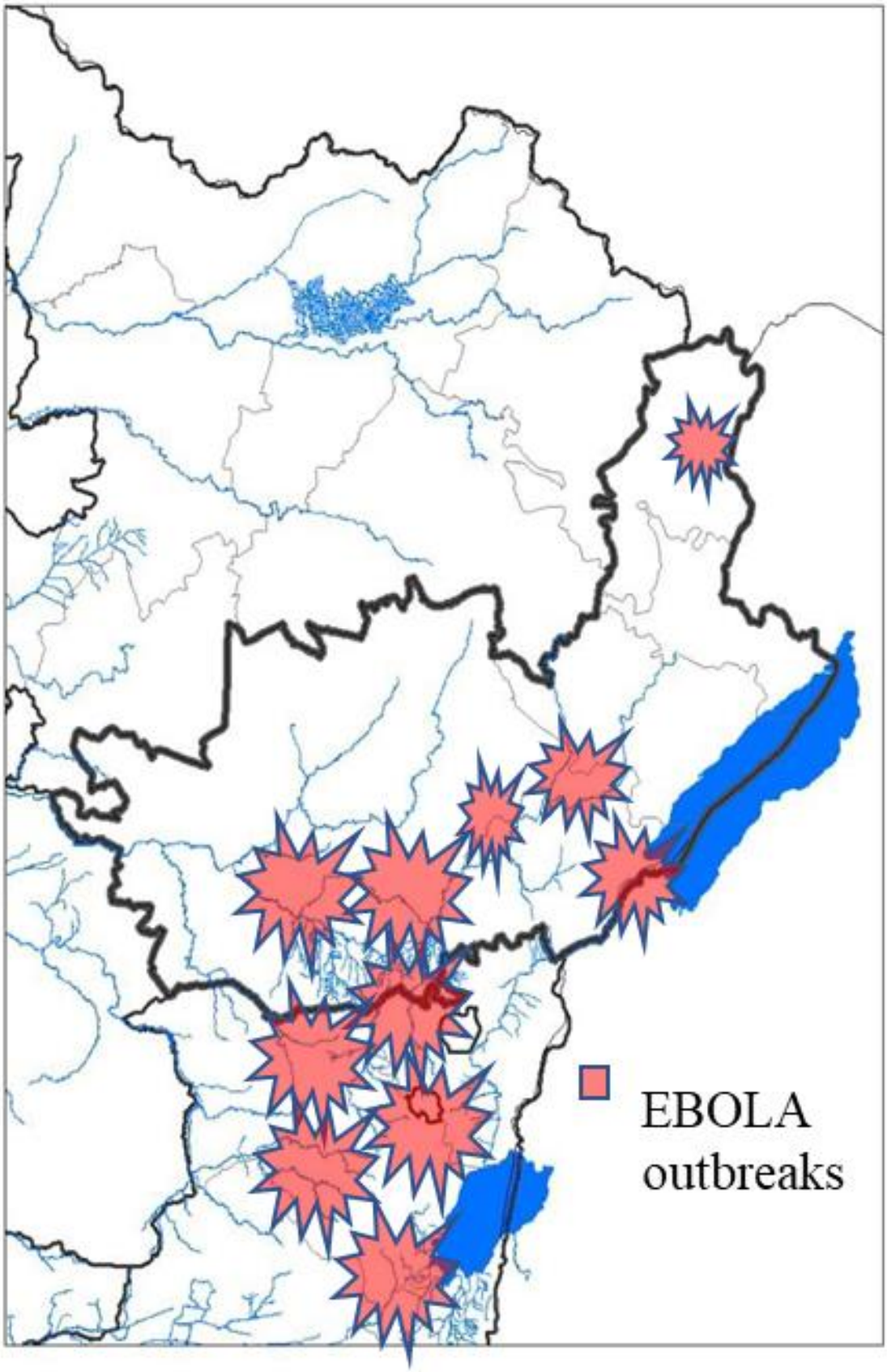


Figure 1.6: Ongoing EBOLA epidemic.

1.8 Goals, and objectives of this thesis

The goal of this PhD thesis is to enhance our understanding of the current burden of intestinal schistosomiasis and the environmental, geographical, socioeconomic, and behavioural factors that underlie its transmission and its spread across the Ituri province. This basis of knowledge will facilitate the establishment of an efficient schistosomiasis control programme in the Ituri province.

In this thesis, we have pursued five specific objectives:

1- To provide an update of current relevant achievements in the field of schistosomiasis diagnosis.
2- To describe the context in which schistosomiasis is transmited.
3- To describe the geographic distribution of *S. mansoni* infection and intensity in Ituri province, northeastern DRC, and to identify key risk factors of *S. mansoni* infection.
4- To determine the infection burden of *S. mansoni* in Ituri province, DRC, and report on its association with morbidity.
5- To closely report severe cases associated with schistosomiasis with a focus on their clinical consequences and management.

2 Approach and Methods

2.1 Framework of the thesis

For the first work packaged document, we first explored the available diagnostic tests for the diagnosis of schistosomiasis in poor endemic settings. For this purpose, we conducted a literature search on Pubmed database from January 1990 to November 2019. More emphasis was put on literatures involving the development of point-of-care diagnostics for parasitic infections. Then, the cited articles were collected by cross-referencing online keyword searches (‘‘*Schistosoma* diagnos- tics methods’’ ‘‘Schistosomisis’’ ‘‘*S. mansoni S. haematobium S. japonicum*’’ ‘‘POC’’). The publications that we perceived as most relevant were solely presented in the review.

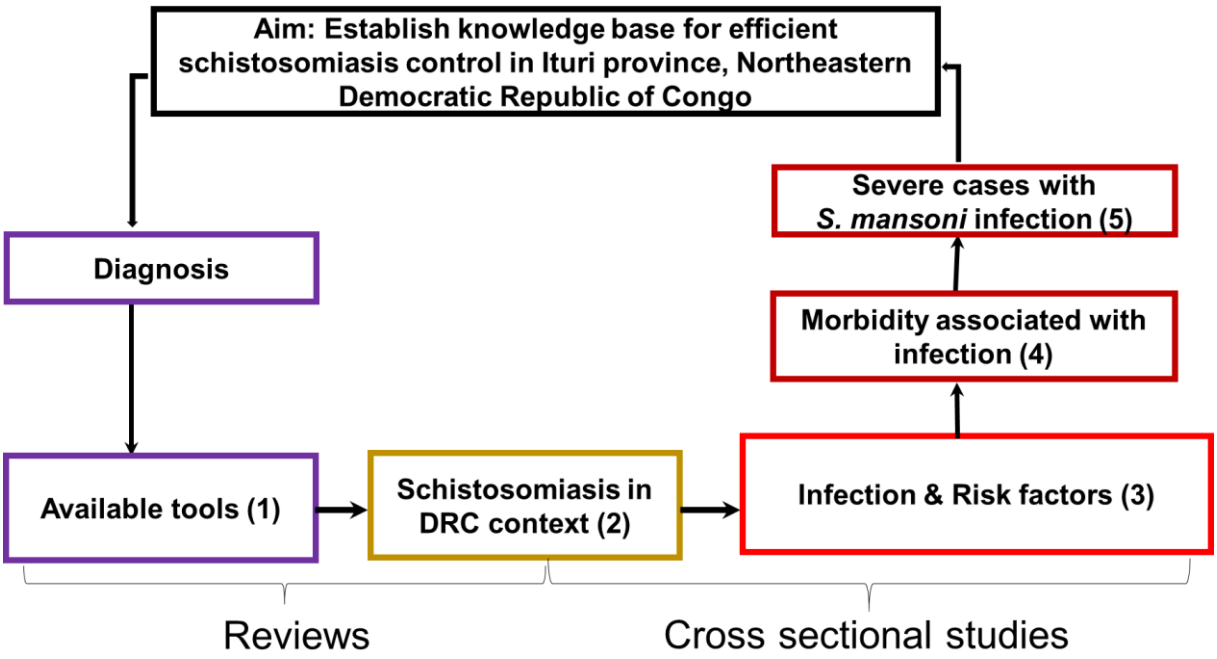
The second work packaged document included collection of both geographic, demographic, socioeconomic, and political environment information surrounding surveys done in 2016 and 2017 in the Ituri province. Both prevalence, morbidity and risk factors of *S. mansoni* infection were explored, analysed and interpreted.

Data over prevalence, intensity and risk factors of *S. mansoni* infection from the three surveys conducted in 2015, 2016, and 2017 were selected and analysed. Then, results from these three surveys were compared and interpreted in the third work packaged document.

For the fourth work packaged document, data were only collected on 2017 from participants who were 6 years old or older, providing at least one stool sample and a urine sample, and who had no missing data of concerning both household and individual questionnaires, anthropometrics, parasitology, clinical examination, and abdominal ultrasound. Information about morbidity and the related risk factors were collected, explored, and their association with *S. mansoni* infection was highlighted.

The fifth work packaged document is based only on the 2017 survey data of 2017. As for the fourth work, only participants aged 6 years or older, who had provided at least one stool sample and a urine sample, and who had no missing data and who were found with severe intestinal and hepatosplenic morbidity wre included. A detailed description case by case was realized.

The approach used for this thesis is summarized in **Figure 2.1**.



[1] Manuscript 1: Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology rapid field testing for clinical management and epidemiology. **Chapter 3**.

[2] Manuscript 2: Schistosomiasis in Eastern Congo: A major neglected healthcare concern in a setting of war and unrest, extreme population poverty, extreme richness in minerals, and minimal infrastructure. **Chapter 4**.

[3] Manuscript 3: Epidemiology of Schistosomiasis in Ituri province, Northeastern Democratic Republic of the Congo. **Chapter 5**.

[4] Manuscript 4: Morbidity associated with *Schistosoma mansoni* infection in Northeastern Democratic Republic of the Congo. **Chapter 6**.

[5] Manuscript 5: Patients with severe intestinal and hepatosplenic schistosomiasis mansoni in the Ituri province, Democratic Republic of the Congo. **Chapter 7**.

Figure 2.1: Framework of the PhD thesis research work.

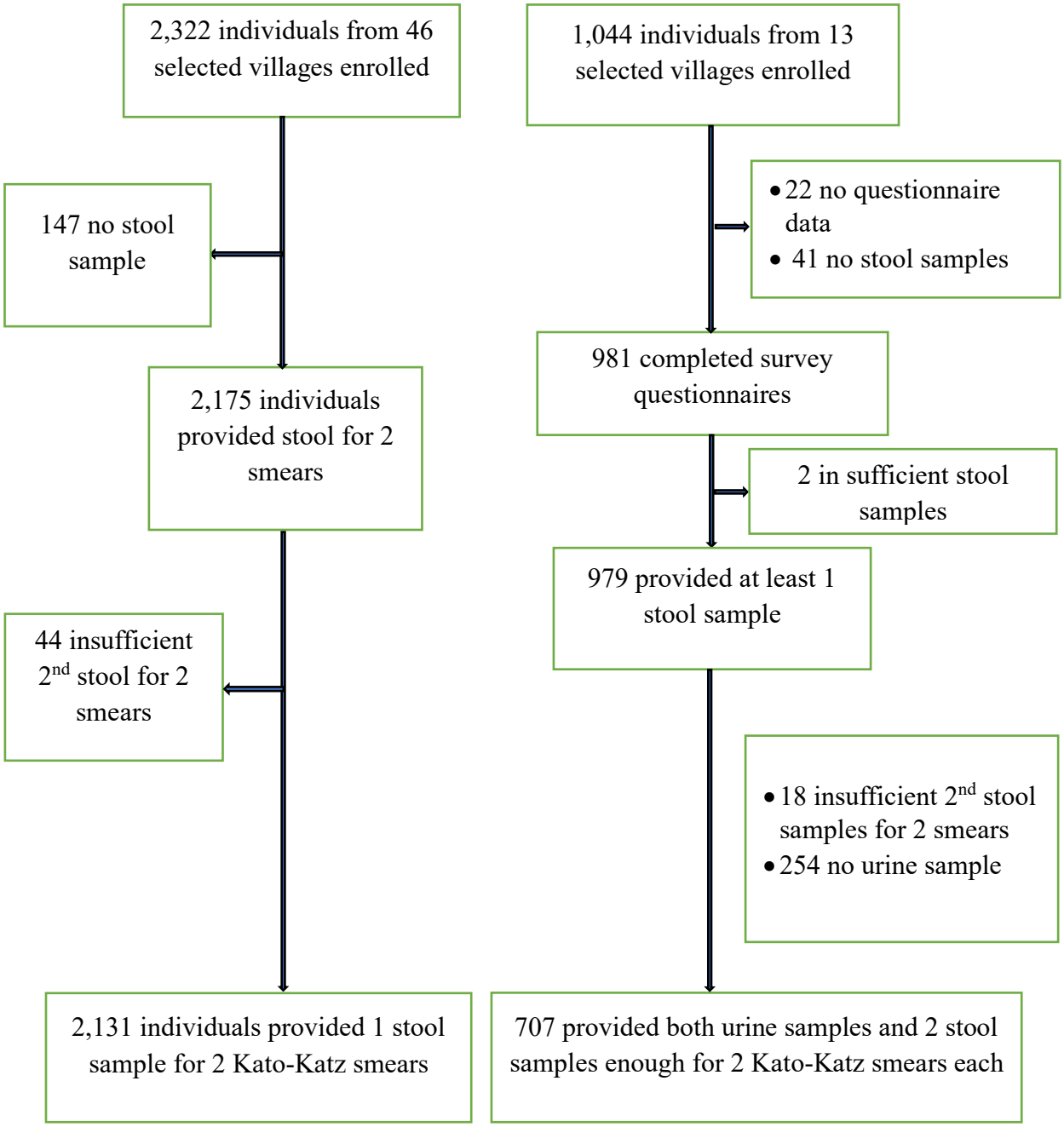


Figure 2.2: Study participants' inclusion flowchart of the 2016 distribution and 2017 in-depth studies.

2.2 Ethical considerations

This study was conducted in accordance with applicable laws and regulations including the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP) and the ethical principles that have their origin in the Declaration of Helsinki. The Swiss National Ethics Committee (EKNZ) approved the protocol and informed consent approval. The approved protocol and informed consent statement were submitted to the Ethic Committee of the University of Kisangani (ECUK, DR Congo) for reviewing. It was only after approval of the protocol by the ECUK, that the investigation started. Prior to any participant enrolment and any study procedures, the participant or tutor was asked to sign or thumbprint and date the informed consent assent. More details are in the method sections of each chapter.

2.3 Study area

Our study took place at the Ituri province, north-eastern DRC. This province of about 65,658 km² is characterized by strong geographic and demographic variability. It has a population of 5.3 million people from about 40 tribes, grouped into the five ethnic groups of DRC. The region has a wealth of minerals but is hard hit by conflicts and instability over two decades. The province borders Uganda Republic and South-Sudan Republic in the North, and three other provinces of DR Congo, namely Nord-Kivu province in the South, and Tshopo and Haut-Uélé provinces in the West. Water bodies are highly abundant, e.g. with about 160 km of shoreline with Lake Albert and Semliki-Albert Nile in the East.

The Ituri province is divided into 5 counties ("territoires") namely Aru, Mahagi, Djugu, Irumu, and Mambasa (Figure 2.4), 42 chiefdoms ("chefferies" or "secteurs"), which are subdivided into several groups ("groupements"), then into villages. It has an estimated population of 5.282 million people, a density of about 80 inhabitants per km², and an estimated growth rate of 3.0%. In 2015, population distribution by age was: 0 – 11 months (4.0%), 1 – 4 years (14.9%) 5–14 years (29.1%), 15 – 49 years (44.4%), 50 – 64 years (5.4%), and ≥65 years (2.2%).

The three studies were conducted in fifteen of thirty-six health districts (Figure 2.3) of the Ituri province, in the northern-eastern DR Congo, namely Bunia, Nyankunde, Rwampara, Komanda, Lolwa, Mandima, Nia-Nia, Tchomia, Bambu, Rethy, Logo, Nyarambe, Angumu, Laybo, and Adi. Selected villages from these health districts were visited (Figure 2.3).

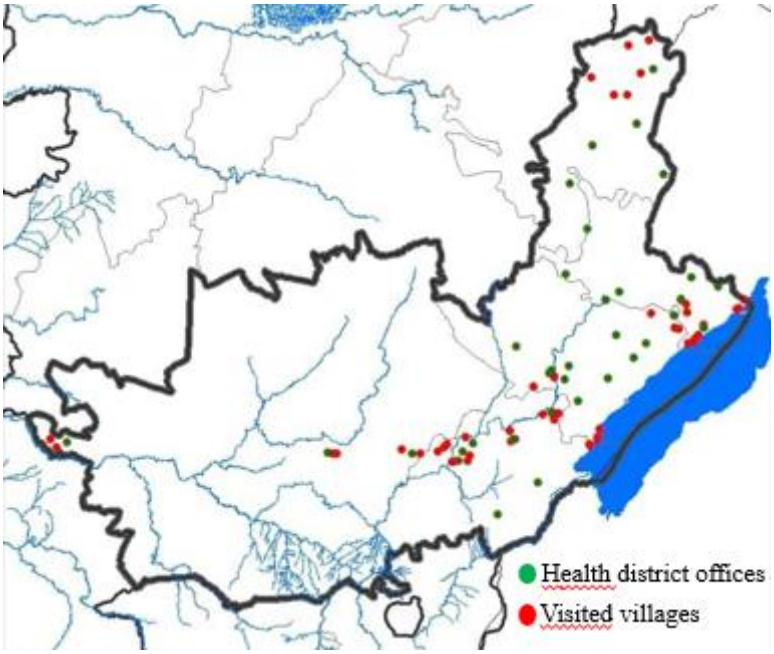


Figure 2.3: The visited villages and the health district offices of Ituri province

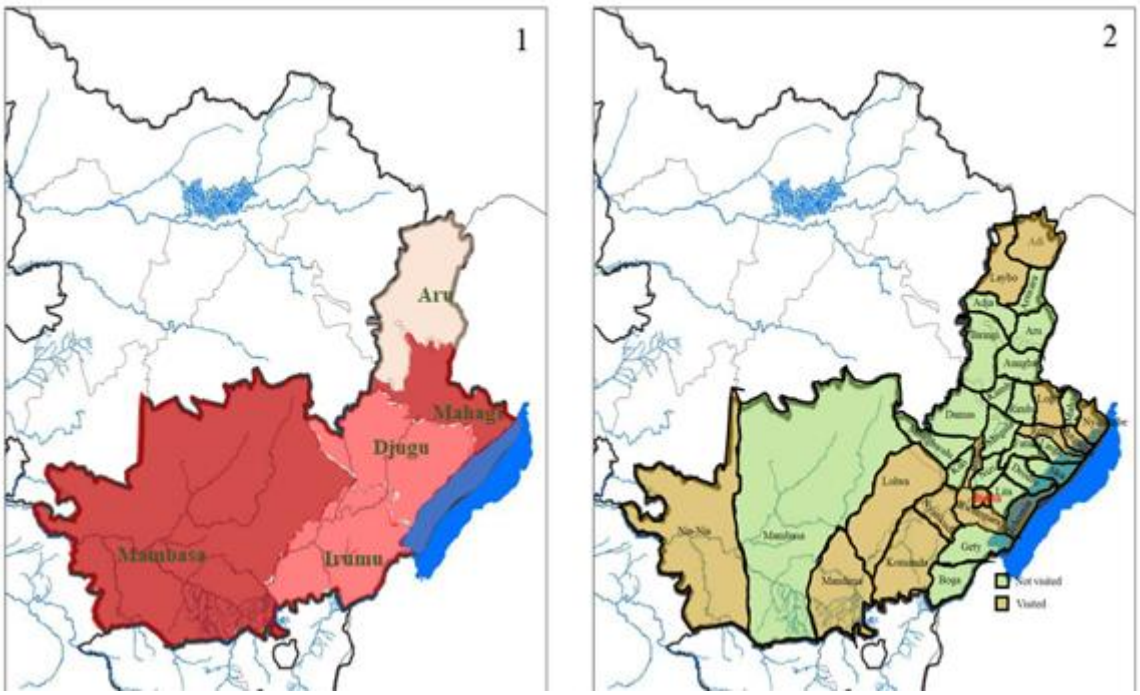


Figure 2.4: 1) the 5 counties ("territoires") and 2) the 36 health districts of Ituri province

2.4 Study design

The major aim of this thesis is to describe *S. mansoni* infection burden in the Ituri province, north-eastern Democratic Republic of the Congo.

Research for the development of this thesis was based on two different works. In order to address the first objective, an internet search was performed to find out the available diagnostic tools. Thus, a scoping review was written.

In order to reach objectives 2-5, three cross-sectional studies were implemented. Firstly, to find out if there was a problem, an explorative field study was planned and carried out among schoolchildren in 2015. Then, for scoping the problem, province-wide and large-scale field study was carried out in 51 villages in 2016. Finally, for assessing morbidity burden, the value of point-of-care circulating cathodic antigen (POC-CCA) test and the socioeconomic background, an in-depth field study was performed in 13 villages in 2017.

Study population was constituted by individuals living in different locations of the province and from the five ethnic groups. The target population was composed of all individuals aged 1 or older and living in the province. Preschool-children aged were included because of the habit of bathing babies in surface waters.

The source population was constituted by individuals 1 year or older and living in the selected areas (health districts and villages) in the province.

A sample was randomly selected from the source population. As there was no census in the country three decades ago and that the population is dispersed over the province, a two-stage sampling was applied. First, as the province is divided into five territories (Aru, Mahagi, Djugu, Irumu, and Mambasa), and into 36 health districts (Figure 2.4), the primary sample units were villages that were drawn in a way that they would represent all the five territories and one third of the total number of health districts. Schools, households and individuals were then randomly selected as secondary sample units.

Study participants were those individuals who voluntarily attended village meeting, signed the informed consent, had their anthropometric measurements done, accepted to provide the requested samples and to undergo clinical and ultrasound examinations.

Based on informal data, villages in which prevalence was ≥39.0% were also randomly selected. Assuming that the population size is big (≥10^6), using 50% as expected prevalence, with a 5% margin error and a design effect equals 2.0, the sample size was then calculated using EPI Info Version 7.2.1.0 (Centre for Disease Control and Prevention, Atlanta, USA) at a confidence level of 95%. A size of 500 individuals would be sufficient. However, more than 500 people were enrolled.

Children below one year were excluded. Household and individual questionnaires, anthropometrics measurements, and laboratory, clinical and ultrasound examinations were performed.

2.5 Fieldwork organisation

The fieldwork was planned as follows: first, we obtained the necessary ethical and administrative authorizations, then we did the sensitization of the expected communities to be visited. After gathering materials and equipment, training of the research team and the methodology of work was tested before the implementation. As mentioned above, field activities were realized. The key elements of the study strategies are summarized in **Table 2.1**.

2.6 Data collection

Before data collection, we checked if the participant has signed the informed consent. Then, respectively anthropometric measurements (weight and height), stool and urine examinations, clinical examinations, and abdominal ultrasound were performed to collect the needed information.

In the visited site, we first organized a meeting with the local authorities. Then the expected participants received detailed explanations concerning the objectives, procedures, expected benefits and potential risks of the study. Detailed information and informed consent sheet in French was read to the study participants by a member of study team and was translated to Kiswahili/Lingala/or to the adequate local language. Their questions were answered. Individual written informed consent was obtained from adult participants and from parents for their children (less than 14 years of age) participating in the study.

All individuals (adults and children) having participated in these studies received 500 mg of Mebendazole (Vermox®) for general deworming in accordance to the DR Congo national

guidelines. Individuals diagnosed with schistosomiasis (*S. mansoni*) were treated with praziquantel 40mg/kg [141].

2.7 Procedures

For schools', villages', and households' visits, we first obtained authorization from Health District officers. After receiving their availability, radio communications were done. On the day of the survey, trained interviewers filled either standardized school or village forms, then individual forms were used [66]. The school form consists of information about the number of pupils (emphasizing girls' number), presence and quality of latrine, presence of water supply, and approximative distance from the nearby body of water. The village form was used to collect information about the habit of using latrine, the main source of drinking water, and the existence of mass drug administration (MDA) for the benefit of the villagers. For the individual questionnaire, information on demographics (age, sex, tribal group), anthropometric characteristics, class level, knowledge on schistosomiasis, previous treatment with mebendazole/albendazole and with praziquantel. We defined the body mass index (BMI) as underweight (<18.5), normal weight (18.5 to 24.9), and overweight (≥25.0).

We categorised infection prevalence as low if it is less than 10.0%, moderate between 10.0 to 49.9% and high if it is equal to or more than 50%. We also categorized infection intensity as light if it is between 1 to 99 EPG, moderate between 100 and 399 EPG and heavy if it is equal to or more than 400 EPG [142], and about 40 mg/kg of praziquantel, using the pole, for everybody diagnosed with schistosomiasis.

Demographic and anthropometric information and Kato-Katz results were entered in the individual forms as source data, and then in computer. Ten percent of Kato-Katz slides were read by the team leader as for quality control [143].

After receiving administrative permission, people were informed by radio about the visit. Once in the village, a meeting was organized with the local authorities. Then, the household to be visited were selected by the local health workers supervised by the community leaders. All members of the selected household were invited to participate.

All participants were invited to an interview conducted using a pre-tested questionnaire. A household-based socio-economic questionnaire was used to collect information concerning household annual income, construction materials, sanitation, water supply, distance to the nearest water body, and what in their view, is the most important health problem in the village. The individual questionnaire focused on demographic, anthropometric, occupational, educational and religion as well as on knowledge, attitude and practices risk factors.

The visited households were in one part from villages located along the shore of Lake Albert and in another part along the main rivers and water bodies of the province. The main inclusion criteria were the expected prevalence, people of both age and sex, and from different socioeconomic and cultural categories (tribe, occupation, education, religion).

The height and weight of participants were measured using Seca analogic bathroom scale and ADE Körper-Analysewaage height rod (Germany). The height and weight were measured to the nearest 0.5 centimetre and 0.5 kg, respectively. Body mass index (BMI) of each participant was calculated as weight in kilogram divided by the square of height in meters (kg/m^2). Following BMI categories were used in our study: <18.5: underweight, 18.5 – 24.9: normal weight, 25.0 – 29.9: overweight, 30.0 – 39.9: obesity, and >40.0: morbid obesity.

Table 2.1: Elements of field studies carried out in Ituri province from 2015 to 2017

Year and focus	Study type	Study strategies			Final sample size
		Sampling	Visiting	Procedures	
2015 (Exploratory)	Cross-sectional pilot survey	Randomly selected schools in 3 health districts	One day visit in the selected school	▪ Recruitment ▪ Informed consent ▪ Anthropometrics ▪ One Kato-Katz	n=435
2016 (Prevalence)	Cross-sectional large survey covering the geographic area	Randomly selected villages in 12 health districts	One day visit in the selected village	▪ Recruitment ▪ Informed consent ▪ Anthropometrics ▪ One Kato-Katz	n=2,131
2017 (Morbidity, risk factors, value of diagnostics)	Cross-sectional in-depth survey	Randomly selected households in 13 villages in high prevalence areas identified in 2016 and 6 health districts	Five days visits in each household within the selected village	▪ Recruitment ▪ Informed consent ▪ Anthropometrics ▪ Detailed socioeconomic parameters ▪ Up to five Kato-Katz ▪ One CCA tests ▪ Clinical examinations ▪ Abdominal ultrasound	n=1,022 Sub-samples n1=979 n2=727 n3=725 n4=707 n5=586 n6=163

Figure 2.5: Procedures: questionnaires, anthropometrics, and collection of samples.

Participants were asked to give approximately 5 grams of first-morning stools on five consecutive days for Kato-Katz test. Appropriate plastic containers were used for this end. From each stool specimen, two slides thick smear of 41.7 mg [79] (Figure 2.7) were prepared and examined by three experienced technicians. For hookworm assessment, microscopic examinations of all preparations were done within one hour. 30 percent of preparations were checked by the principal investigator. All present helminth eggs were counted. Intensity of helminth infection was calculated by multiplying the mean number of eggs found in each slide by 24. The result was expressed as eggs per gram (EPG) of stool [143]. *S. mansoni* intensity was categorized as light: 1 – 99 EPG; moderate: 100 – 399 EPG; and heavy: ≥400 EPG.

On the last day, people who gave stool for at least two days were invited to give approximately 60 ml of urine sample for the detection of the point-of-care circulating cathodic antigen (POC-CCA) of *S. mansoni*. Wide mouth plastic containers were used. Both stool and urine examinations were performed at the village health centre facilities.

Figure 2.6: Duplicate Kato-Katz slides examined by the survey lab team

CCA test was performed according to the manufacturer's guidelines Rapid Medical Diagnostics, Pretoria, South Africa) [144]. Selected participants were asked to provide first-morning urine samples for this end. When the test is postponed for the next day, urine samples were kept at 2 – 8ºC in Solar fridge. Test results were considered as negative when the CCA band did not appear within 20 minutes. Both traces, weak, medium and strong coloured CCA bands were scored as positive results. Questionable results were discussed between at least two technicians and the principal investigator (Figure 2.7).

2.8 Data analysis

The overall data were double entered in Microsoft Excel software (Microsoft, USA) and after validation, they were transferred on Stata 14 software (Stata Corp, College Station, USA). Only, participants with complete household and individual questionnaires and with at least two stool examinations were retained for the analysis. The prevalence of infection was expressed as the number of positive individuals divided by the total examined. The infection prevalence was then presented as a smoothed age prevalence curve by the mean age and gender of participants. The intensity of the infection was estimated as helminth egg counts per gram of stool (EPG) examined by Kato-Katz test [79]. The intensity thresholds were classified as light, moderate, and heavy infections [66]. Presence of schistosomiasis, demographic, anthropometric, occupational, socioeconomic, domicile, household, stay duration, health district, village, tribe group, religious, clinical, ultrasonographic, geographic, environmental, knowledge, attitude and behaviour characteristics were considered as categorical variables and presented as frequencies and percentages. Arithmetic mean intensity was calculated. Results obtained using Kato-Katz and point-of-care circulating cathodic antigen (POC-CCA) tests were combined. An univariate analysis of demographics (age and gender), residence (domicile, household, village, health district), environmental (proximity to bodies of water), geographical (altitude), socioeconomic (household yearly income), behavioural (washing clothes, swimming, fishing, farming, cleaning motorcycles) co-variables was performed to find out the association with *S. mansoni* infection status and a logistic regression analysis was carried out to identify the potential risk factors. Pearson's chi-square (χ^2) test was used to examine the differences between the frequency distribution. Odd ratios (OR) and 95% confidence intervals (CI) to determine whether or not there are associations between categorical variables. Multiple logistic regression analysis was then used to identify the most significantly associated factors with schistosomiasis. All tests were considered significant at $P<0.05$.

The primary end point was the epidemiological descriptors of schistosomiasis in Eastern DRC, broken down into demographic, geographic, socioeconomic, and behavioural parameters.

The secondary end points were comparison of the sensitivity and specificity (false negatives, false positives) of one duplicate Kato-Katz [79] test with multiple (2 to 5) duplicate Kato-Katz test used for the diagnosis of schistosomiasis, and the comparison of the sensitivity (minimal detectable amount of parasites) of the point-of-care circulating cathodic antigen (POC-CCA) [145] assay with microscopy used for the diagnosis of schistosomiasis parasites.

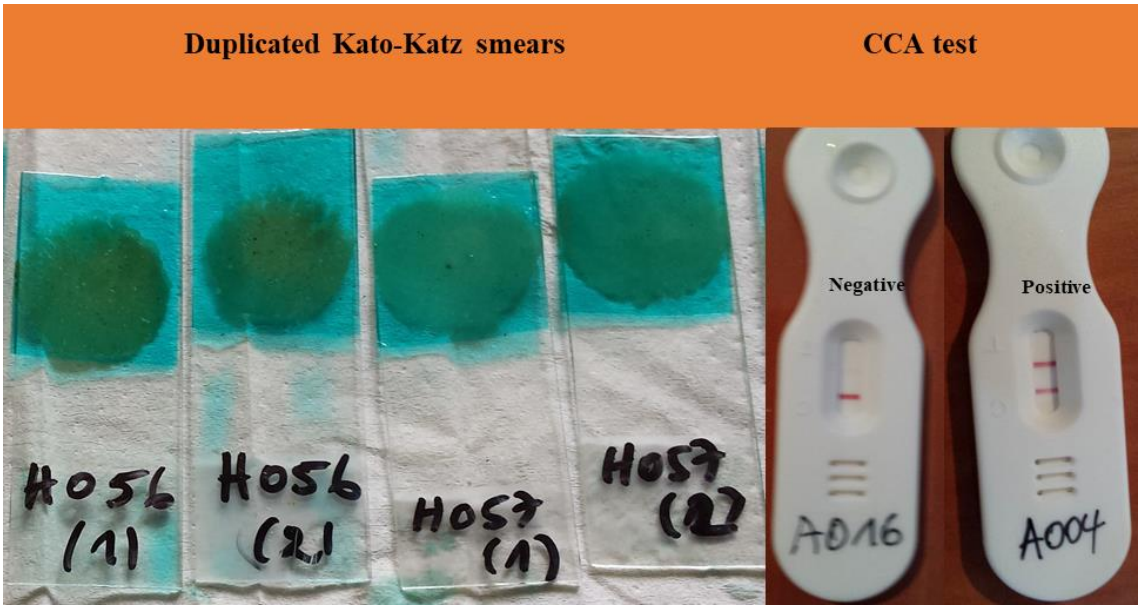


Figure 2.7: Used tests: Kato-Katz duplicated smears and point-of-care circulating cathodic antigen (CCA) – showing and negative (left) and positive (right) test results, respectively.

3 Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology

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Short title: Sschistosomiasis emerging diagnostic tools.

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This review has been published in *Precision Nanomedicine*, 2020 (3) 1: 439-458.

3.1 Abstract

Schistosomiasis is a neglected invasive worm disease with a huge disease burden in developing countries, particularly in children, and is seen increasingly in non-endemic regions through transfer by travellers, expatriates and refugees. Undetected and untreated infections may be responsible for persistence of transmission. Rapid and accurate diagnosis is the key for treatment and control. So far, parasitological detection methods remain the cornerstone of schistosoma infection diagnosis in endemic regions but conventional tests have limited sensitivity, in particular in low-grade infection. Recent advances contribute to improved detection in clinical and in field settings. The recent progress in micro- and nano- technologies opens a road by enabling the design of new miniaturized point-of-care devices and analytical platforms, which can be used for rapid detection of these infections. This review starts with an overview of currently available laboratory tests and their performance and then discusses emerging rapid and micro/nanotechnologies-based tools. The epidemiological and clinical setting of testing is then discussed as an important determinant for selection of the best analytical strategy in patients suspected to suffer from schistosoma infection. Finally, it discusses the potential role of advanced technologies in the setting near to disease eradication is examined.

Key words: schistosomiasis, rapid, accurate, POC, diagnosis, immunoassays, PCR, LAMP, microfluidic, microarray.

3.2 Introduction

Schistosomiasis is one of the most prevalent and widespread [146] parasitic Neglected Tropical Diseases (NTDs) in the world. It is caused by fluke worms of the genus Schistosoma and affects human and various other animals [47, 147]. The six species involved in human infections are *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. guinensii* and S. intercalatum. The respective distribution, the population at risk and the main clinical manifestations are summarized in Table 1.3. Infection starts when cercariae released by snails penetrate through the skin exposed in infested water [148]. More details on the life cycle are given in Fig 1. Schistosomiasis affects 221 million people where children aged between 5-14 years represent 45.8% of the affected patients [149] and puts at risk 800 million people in 74 countries worldwide [41, 150]. In sub-Saharan Africa the number of affected people is 54 million and

393 million are at risk (WHO, 2019). Worldwide, the disease kills annually about 300,000 patients [151] and results in approximately 25 million disability adjusted life years lost [152] despite implementation of control measures [15].

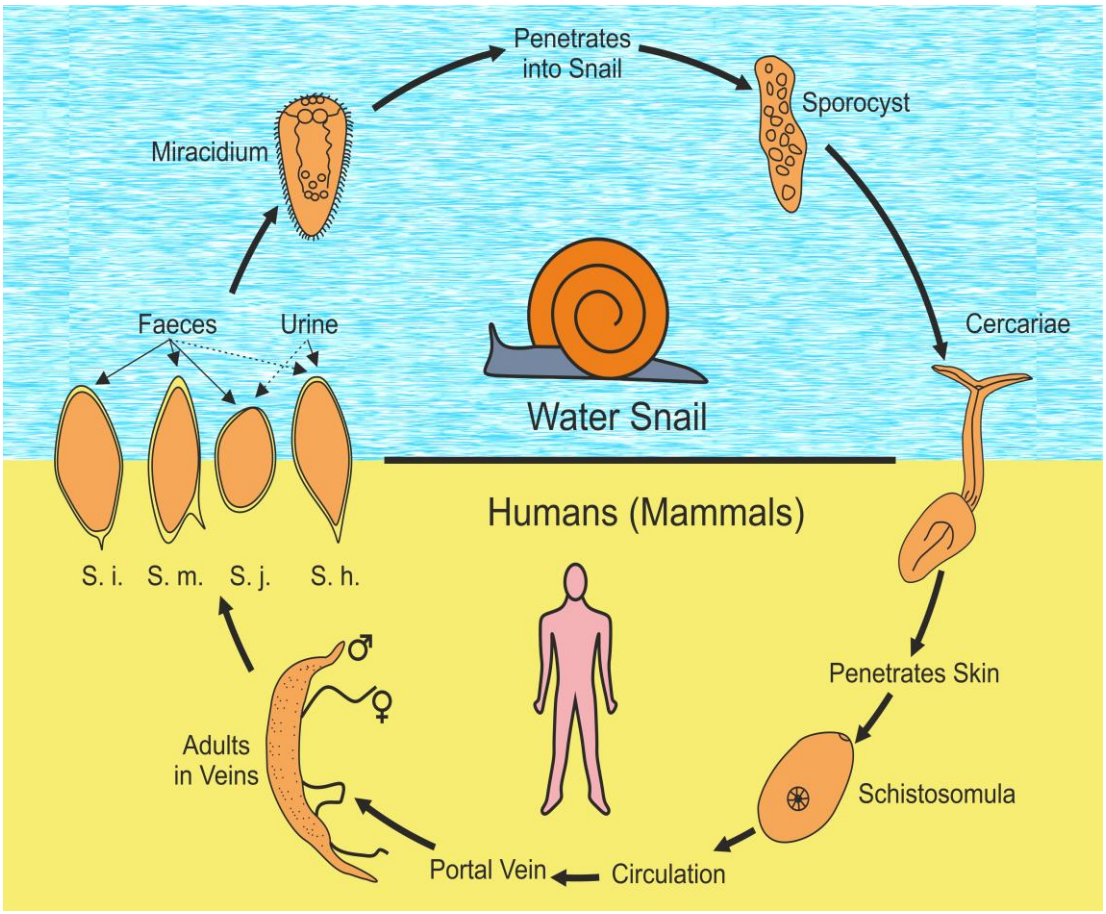

Figure 3.1: *Schistosoma mansoni* life cycle (1) Paired adult worms (female is thin, male is bigger), (2) Eggs (see note below), (3) Ciliated *miracidium*, (4) *Biomphalaria* intermediate host snail, (5) Sporocyst, (6) *Cercariae*, the infective stage, and (7) Schistosomula, the young parasite.

Selective therapy of school children or mass drug administration (MDA) are widely used to control schistosomiasis, but fail to prevent rapid reinfection in endemic areas [151]. A substantial decrease of morbidity and mortality is observed in some of these areas, but the disease continues to spread to new geographic regions. It is increasingly imported into non- or low-endemic countries due to the increased migration of human population (e.g. refugees, international tourism, and/or international projects and additionally, environmental changes that result from development of water resources and population growth and migration can facilitate the spread of schistosomiasis [35, 153]. Diagnostics address both the individual and population needs. At individual level they first can detect a disease and identify patients at risk of severe disease, which will permit good clinical decision making and good case management.

The use of diagnostics also supports: 1) the detection and prevention of drug resistance, 2) the surveillance of a disease, and 3) the assessment of the efficacy of drugs and vaccines in clinical studies [76]. Rapid and accurate diagnosis of schistosomiasis is a challenge [97]. It remains a key requirement for treatment and control [38, 154-157]. There is still a need to determine the existence of infection in a simpler and more reliable fashion, both, in hospital settings where severe pathological effects are suspected, as well as in the field [54, 158].

Current diagnosis of schistosome infection is based mainly on clinical symptoms, and therefore low-level and chronic or asymptomatic/atypical infections are often missed [57, 102]. Various procedures to detect this infection have been validated and include direct parasitological techniques, immunologic methods, and molecular approaches [107]. The sensitivity of parasitological tests decreases when parasite egg numbers are low or eggs are absent [78] and specificity of rapid diagnostic tests (RDTs) is variable [107]. Routine serological tests as enzyme-linked immunosorbent assay (ELISA) have good sensitivity but may cross-react with intestinal nematodes [159]. Methods based on the amplification of a highly repeated deoxyribonucleic acid (DNA) sequence by polymerase chain reaction (PCR) in faecal and serum human samples [110] show high sensitivity, high specificity and good predictive value. Yet, the test cost and the need for specific laboratory equipment and conditions limit the use of these assays in many laboratories. The real-time polymerase chain reaction (RT-PCR) method, in spite of its high sensitivity and specificity [107], has remained only as a confirmatory diagnostic test, since it is intensive work and expensive. To reduce the costs Loop Mediated Isothermal Amplification (LAMP) of DNA was developed. Both LAMP and microarray methods have been tested and so far these methods promise to be excellent tools in epidemiological and clinical screenings [146] but, the diagnostic performance of these

techniques in areas with different schistosomiasis prevalence is so variable that it is difficult to declare a “gold standard” test [98, 99]. In addition, the amplification mechanism of LAMP may easily lead to carryover contamination and therefore false positive results.

The rapid evolution of micro- and nanofabrication technologies opens a door for development and evaluation of new methods and tools for the diagnosis of schistosome infection. In this chapter we will first present the current available diagnostics, followed by the latest developments in the field and end with a discussion. With a view on clinical application, the value of diagnostic tests for patients in different epidemiological scenarios is discussed. As a comprehensive review of the huge body of work that is being performed is out of scope of this paper, we aim to provide an update of current relevant achievements in the field. The cited articles are collected by cross-referencing online keyword searches (‘‘*Schistosoma* diagnostics methods’’ ‘‘Schistosomisis’’ ‘‘*S. mansoni S. haematobium S. japonicum*’’ ‘‘POC’’) in citation and database searching on Pubmed from January 1990 to November 2019. The works perceived as most important are exemplarily presented in this review. The emphasis of literature citations is on the development of point-of-care diagnostics for parasitic infections.

3.3 Established diagnostic assays for schistosomiasis

Current laboratory tests for schistosome infection are based on direct parasitological detection, the detection of circulating antigens and/or specific antibodies, and on molecular methods. More detailed information can be found in focused literature [70, 76]. As the background of evaluation for emerging and future test modalities, the current clinically available tests are summarized in the next paragraphs.

Microscopy: direct detection of parasite eggs

The reference method for the laboratory diagnosis of a schistosome infection is parasite egg detection in the infected individual's stool, urine, or tissues [48, 77]. Such parasitological detection (PD) is widely used because it is relatively efficacious and moderately cost effective for case-management, screening or surveillance. The success of control measures against schistosomiasis, has been difficult to determine because the sensitivity of PD decreases when there is no egg excretion, or it diminishes, principally in areas of low endemicity [78].

Figure 3.2: Current diagnostis of intestinal schistosomiasis relies on the microscopic detection of S. mansoni eggs in stool samples, a laborious, time consuming process relying on infrastructure (microscopes) and specific expertise. Low-intensity infections are frequently missed.

Other direct parasitological tests for intestinal schistosomiasis are the Kato-Katz (KK) [79] technique, the miracidia hatching test (MHT) [160], the salinity gradient [161] and on the recently developed FLOTAC [80-85] and Helmintex assay [86-91]. The K-K technique has the advantage of egg quantification in fresh stool samples. An amount of approximately 41.7 mg or 50mg of faeces is placed in a 1.5mm*6mm template hole. Egg number is then counted and extrapolated to 1g [92, 162], yielding infection density (Table 2). This method is still recommended by the WHO for diagnosis at the community level. MHT can be done using the miracidia hatching device [163] or by sieving stool sample through a nylon tissue bag for concentrating the eggs. Then, hatching is carried out in a well-lit room at a temperature of 25±3ºC followed by counting of the swimming miracidia [160]. The FLOTAC technique is more sensitive when compared to the KK technique, but it requires specific equipment, flotation solution, a suitable preservative medium that can affect the outcome of the test [85]. The principle of the Helmintex test is based on conjugates that are formed between the magnetic particles and the iron that is present in the eggshell pores of *S. mansoni*. Factors such as electrostatic forces or the surface ornamentation of the eggs play also a role [164].

In urinary schistosome infections, direct microscopy of filtered urine is used. After gentle homogenization, 10 ml of urine is filtered through a membrane on which the number of eggs is counted in the microscope at 40X magnification [165]. These methods are still considered as reference methods [93], in spite of their limited capability to identify schistosome infection consistently in low endemic areas. Development of better diagnostic tools for case-management and for disease control is therefore required [76].

Table 3.1: Expression of the intensity of schistosome infection [165].

Types of Schistosomiasis	**Intensity expression**	**Light**	**Moderate**	**High**
Intestinal schistosomiasis	Eggs per gram of stool (EPG)	1 – 99	100 – 399	≥ 400
Urogenital schistosomiasis	Eggs per 10 ml of urine	< 50		≥ 50

Parasite-derived material detection

Adequate differentiation between past and current infection requires assays, which detect circulating parasite antigens. The existence of such antigens was first described by [166-177], and an inventory has been published [178]. The most thoroughly investigated antigens are the circulating anodic antigen (CAA) [179], a gut-associated proteoglycan (GASP) [180] and the

circulating cathodic antigen (CCA), originally named the M-antigen [181], which is a polysaccharide antigen emanating from the worm gut [182]. They are named relatively to their electrophoresis migration [183]. Their presence in patients corresponds well with active infections [168, 173]. Detection of circulating antigens could be used as well as for evaluation of chemotherapy efficacy [150].

Other antigens as targets for diagnosis have been reported: schistosome adult worm antigen (SAWA), schistosome egg antigen (SEA), enzymes, parasite proteins and tegument antigens; they can be detected in patient serum [111, 184]. As some antigens are cleared by kidneys, urinalysis can produce evidence of their presence in schistosomiasis due to *S. japonicum* [185], *S. haematobium* [54] and *S. mansoni* [186]. Antigens from virtually all stages of the schistosome life cycle have been tested for immunodiagnostic potential and methods for their direct detection in blood, stool or urine has been developed. Methods for detecting circulating antigens generally involve the capture of the antigen by a monoclonal or polyclonal antibody, with specificity for repeated epitopes on the antigen. In most cases specific monoclonal antibodies are used [185]. Examples of these immunoassays are ELISA [77], radio-immuno-assay (RIA) [96, 187], and direct fluorescent antibody tests [188-190].

Lateral flow immuno-chromatographic assays or "Rapid Diagnostic Test" (RDT) are used for diagnosing antigens in serum [191], and in urine samples [192, 193]. They provide rapid results, are a non-invasive technique and are easy to use because they are typically cassettes or dipsticks [94, 95]. Details on technical procedures can be found in the cited literature [144]. Figure 2 shows a RDT for CCA in urine for detection of S.mansoni. RDTs permit detection of active infections and are efficient in testing of large numbers of samples. However, they are frequently false negative due to inhibitory host antibodies [96] and often cannot distinguish between infections of different species because they are not species specific [97]. Diagnostic specificity of these tests is high because few false-positive are found outside endemic areas and circulating antigen levels generally correlate with excreted egg counts [194-196]. AgD assays need to be evaluated further and are not currently considered as suitable replacement for traditional diagnostic tests [98, 99].

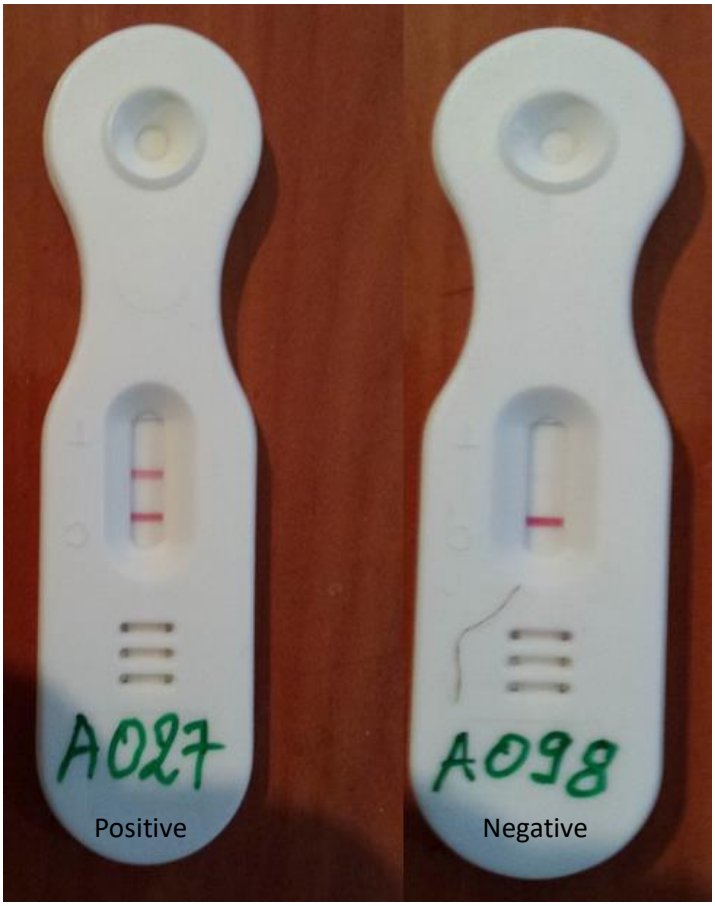

Figure 3.3: Rapid diagnostic test for circulating cathodic antigen allows point-of-care diagnosis of schistosoma infection. Field studies have shown very good sensitivity when prevalence and parasite load is high, but sub-optimal sensitivity for low grade infection.

Antibody detection (AbD)

Schistosoma infection activates specific immunoglobulin-dependent responses against several parasite antigens [100]. Most AbD assays measure serum immune reactivity to SAWA and SEA preparations for diagnostic purposes, using crude extracts, or parasite tegument proteins. AbD assays exhibit a modest sensitivity and a limited specificity although several antigens are available for diagnostic purposes, and some studies have shown their superiority to parasitological methods in low endemic areas (LEAs) [101]. Nonetheless, AbD is more attractive for monitoring areas of controlled transmission and are important for the diagnosis of atypical forms like schistosome neuro-infection [57, 102]. Screening and diagnosis should rely on the use of two or more immunological tests to improve sensitivity because the test result depends on worm burden.

AbD assays include various forms of RIA and ELISA, indirect immunofluorescence (IIF), complement fixation (CF), indirect agglutination of erythrocytes or other tests, of which each have their own specific weaknesses [197]. Many of these diagnostic techniques were miniaturized. Detection of antibodies in patient serum or cerebrospinal fluid (CSF) became possible [58]. Most AbD assays exhibit high sensitivity; however, they show limited specificity due to crude antigen preparations. They allow early diagnosis before oviposition of the worm [198, 199]. With the interpretation of the results one has to take into account a possible persistence of host antibodies after the patients cure [104, 200].

Alternative methods

Haematuria and proteinuria are associated with *S. haematobium* infection and are therefore often used for indirect diagnosis or as screening tool for schistosome infection [104, 105]. The cheap urine dipsticks for macroscopic haematuria and proteinuria detection are being used to guide treatment and public health interventions for *S. haematobium*. Haematuria is relatively specific for infection in a *S. haematobium* endemic area. However, haematuria has a variable sensitivity [106].

Molecular approaches

Although parasitological detection methods have proven to be inadequate as a gold standard, advances in the development of antigen and antibody detection assays are not sufficient to allow their use either universally or as reliable single detection approach in schistosomiasis diagnosis,

particularly in areas of low endemicity [107]. To overcome the shortcomings of both parasitological and immunological methods, development of more sensitive and more specific molecular diagnostic tools diagnosis is desirable. The availability of DNA detection techniques may evolve to potentially valuable tools in the diagnosis of a schistosome infection. DNA-based assays have also proven to be useful for cure assessment [107]. The PCR technique has shown its clinical value in a wide variety of infectious diseases and in a variety of sample types including blood, urine and saliva [108]. It has been successfully used for the sex determination of the cercariae in *Schistosoma* sp. studies and in the development and application of new techniques to generate expressed sequence tags. Hamburger et al. [201, 202] developed a PCR protocol that was based on amplification of a highly repetitive DNA sequence for monitoring *S. mansoni* infestation in water-based on DNA sequences from *S. mansoni* cercariae. According to Reithinger et al. [109], PCR assays can be divided into three distinct formats: namely the mid-tech approach, represented by conventional PCR, the high-tech represented by real-time PCR and the low-tech, represented by loop-mediated isothermal amplification (LAMP). PCR-based methods are considered to be highly sensitive and a detection limit is 1fg of *S. mansoni* of genomic DNA has been reported [107]. PCR can be performed on faeces, urine or serum [110-112]. In other studies, the use of PCR has been reported for diagnosing female genital *S. haematobium* infection and low intensity of *S. japonicum* infections in stool samples [203, 204], and for detection and quantification of *S. mansoni* and *S. haematobium* when present with other parasitic co-infections [205]. Superior accuracy, sensitivity and specificity in areas with a low intensity of infection have been observed using antigen detection by PCR-based methods. It can be used to efficiently detect an active infection in almost 60% of IgG positive individuals who do not excrete eggs [112, 206]. Different studies demonstrated that the absence of amplification of other helminth DNA in a PCR assay is a strong indicator of its species-specificity [103]. Thus, PCR is more sensitive than the Kato-Katz technique. However, Gomes and Enk [112, 207] found different results in samples tested by conventional PCR and microscopy. They observed that up to 41.6% of samples negative by KK are positive by PCR and that PCR assays for *S. mansoni* diagnosis increase prevalence estimates to above 38%, whereas positivity on K-K assay ranges from 18% to 30.9%. A high degree of specificity may render it preferable to serological techniques. Even though these studies have demonstrated that PCR-based technologies are reliable, specific and sensitive tools, they are not widely used in low-income countries because highly skilled personnel and expensive cyclers are needed. Use of thermocycler machines to amplify DNA is severely hampered in field situations where electric power is unstable, intermittent or absent. There is a real need to render these techniques

easier to use and technically more robust, such that obvious advantages of these tests can be translated into broad clinical utility even in resource-constrained locations [69, 208].

The value of standardized quantitative PCR-based methods for the detection of Schistosoma infection was shown in experimental settings [198]. RT-PCR was found highly sensitive and specific in detection of active schistosomiasis [103]. Wichmann [209] found that blood-based RT-PCR detected 95% of true positive patients when, in contrast, anti-schistosome antibodies and microscopy were positive only in 72% and 25% of cases respectively. Moreover, RT-PCR also permits the determination of infection intensity in samples with a low parasite burden. Obeng [210] examined urine specimens from children to detect circulating cathodic antigen, and then performed RT-PCR on urine sample after storage at -80ºC using internal-transcribed-spacer-2 (ITS2) sequences for *S. haematobium*. When compared with eggs in the urine, the RT-PCR test was 100% sensitive, yet specificity was low. The detectable product was dependent on the number of eggs passed in the specimen and the authors postulated that the template DNA was derived from the eggs.

Currently, all PCR variants demand more sophisticated laboratory equipment and a greater operational effort when compared with the K-K technique, in terms of low costs and ease of operation [110].

New DNA detection using new technologies, such as LAMP (Figure 3), have been developed. LAMP amplifies a few copies of DNA to 10^9 copies in less than an hour with high specificity and efficiency under isothermal conditions. LAMP method employs a *Bacillus stearothermophilus* (*Bst*) DNA polymerase and a set of four [211] to six or more specially designed primers: inner forward primer (FIP), inner backward primer (BIP), outer forward primer (F3), outer backward primer (B3), loop forward (LF) and loop backward (LB) that recognize a total of six distinct sequences on target DNA. A standard LAMP reaction consists of a large amount of reaction components, namely the enzyme and its buffer, three sets of primers of varied concentrations, and a detection dye. Significant progress has been made with modification of the LAMP method. The LAMP reaction produces large amounts of magnesium pyrophosphate ($Mg_2P_2O_7$) and double-stranded DNA (dsDNA), avoiding post DNA amplification manipulation. LAMP has shown high sensitivity, detecting 0.08fg of genomic DNA [212]. The LAMP method has the advantage of being able to amplify target DNA from partially processed and/or non-processed samples [213]. It can be conducted in less advanced field laboratories with much less intensive training of local laboratory personnel. When a large

number of examinations are undertaken, the use of LAMP method could save time and financial resources [214]. This method has demonstrated superior accuracy when compared to the conventional PCR in detecting several pathogens such as viruses, bacteria, protozoa in human [215]. In diagnosis of schistosomiasis, LAMP was found highly sensitive for detection of *S. japonicum* infection pre- and post-chemotherapy in experimental settings [216]. LAMP was recently used for the detection of *S. japonicum* in human serum samples. It revealed high analytical sensitivity (96.7%) detecting 0.08 fg of parasite DNA and a specificity of 100% [217]. Fernandez-Soto [146] are leading in development of a sensitive, specific, cost-effective and easy to perform LAMP assay for early diagnosis of *S. mansoni* in faeces. However, the amplification mechanism of LAMP may easily lead to carryover and therefore false positive results.

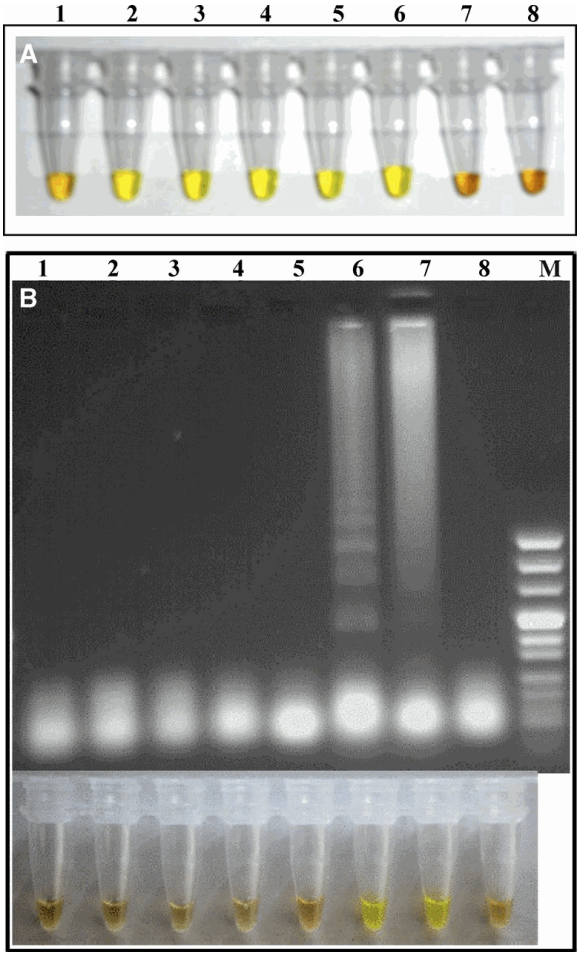

Figure 3.4: Isothermal loop-mediated amplification (LAMP) for detection of schistosoma DNA. Yellow color indicates detection of specific DNA. A1-6: schistosoma positive snails; A7-8: negative controls. B: evaluation of LAMP result with agarose electrophoresis: B1-5,8: negative control. B6-7: schistosoma positive snails. The reduced hardware requirements for LAMP compared to conventional PCR and the simple, colorimetric readout may lead to field-applicable high-sensitivity molecular testing. From Hamburger et al [214].

Table 3.2: Comparison of the main characteristics of diagnostic tests for schistosome infection.

Characteristics						
Test	**Methods**	**Advantages**	**Disadvantages**	**Price US$)**	**On market**	**Reference**
Microscopy	Egg detection	Simple Quantification	Limited sensitivity Laborious	0.4*	Yes	[96]
ELISA; DDIA; IHA	Antibody Detection	Sensitive	Limited specificity	5.0**	Yes	[218, 219]
RDTs	Antigen Detection	Rapid; Large scale testing; Specific	Variable sensitivity; False positives	2.6*	Not yet	[96, 188]
ELISA DDIA*** IHA****	Antigen Detection	Sensitive Specific	Variability in test results		Yes	[220-222]
PCR	DNA detection	Highly sensitive Specific	Laborious; Expensive; Instrumentation; Not quantitative	6.4	Yes	[155, 204]
RT-PCR	RNA detection	Highly sensitive Quantitative	Laborious; Expensive; Instrumentation;	7.7	Not yet	[210, 222]
LAMP	DNA detection	Highly sensitive Accurate	Some Instrumentation, false positive		Not yet	[146, 186, 216, 223]

Ref., Reference * Price without including equipment use and personnel labour [107]
** Estimated price
*** Disperse dye immunoassay (DDIA)
**** Indirect Hemagglutination test (IHA)

3.4 Emerging diagnostic technologies for schistosomiasis

Advances in micro-and nanofabrication technologies have greatly contributed to improving POC laboratory diagnosis [224]. Benefits of miniaturization include low consumption of costly reagents and power, minimized handling of hazardous materials, short reaction times, portability and versatility in design, and capability for parallel operation, all of which are particularly important for POC diagnosis in the tropical and developing countries where schistosomiasis is endemic [225]. Important development in this field are presented below.

Microfluidic platforms

Microfluidic systems, known as lab-on-a-chip (LOC) and micro total analysis systems (μTAS) integrate, in a single chip, several functional modules for specimen processing, biochemical reactions, transportation, and product detection, with on-chip control of thermo-pneumatic pumps, micro-heaters, temperature sensors, miniaturized fluorescence detectors, sample/analyte concentrators, and filters [219]. Microfluidic techniques are increasingly incorporated into diagnostic systems due to the inherent advantages of miniaturization and integration of complex functionality. Actually, magnetic bead-based microfluidic system allows rapid and simultaneous serological analysis of IgG and IgM associated pathogens infection [220, 221, 226].

A compact disk (CD) microfluidic device based on reciprocating flow was built to realize a rapid DNA hybridization assay for nanolitre samples [222] and is shown in figure 4.

Song et al. [227] presented an instrument-free disposable microfluidic POC device for the on-site detection of schistosome parasite infection in blood samples obtained from a finger prick. On board a reaction chamber is present and once filled with water, it is heated to its operating temperature, which is used to incubate a LAMP reaction. The emission of fluorescent light is detected by eye or with the camera of a smartphone. This device has potential, however the discussed crossover contamination when using LAMP needs to be taken into account and thus a second test confirming an infection might be necessary.

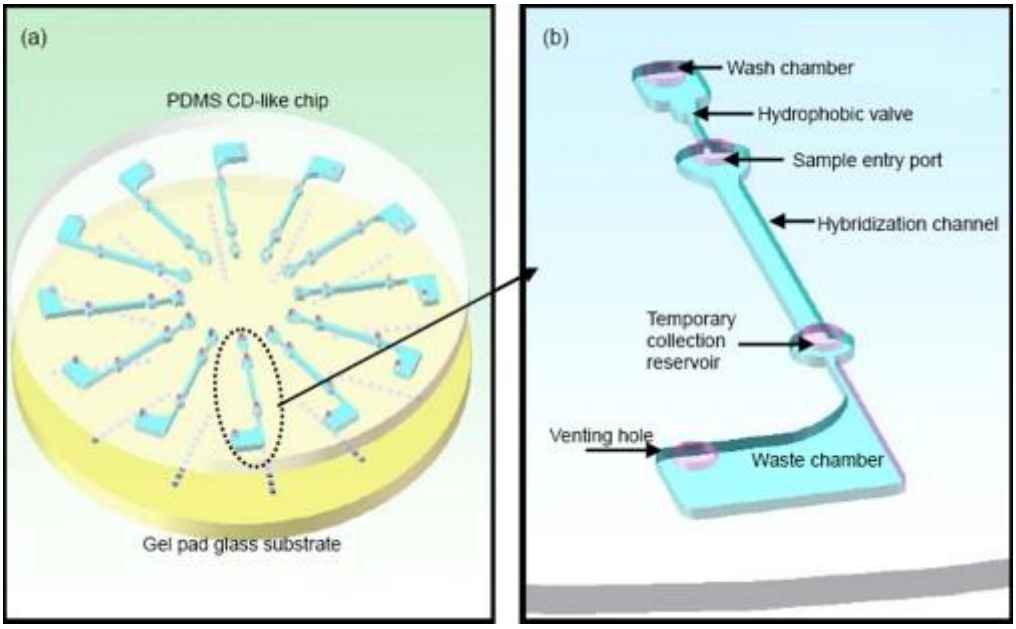

Figure 3.5: A compact disc (CD)-like microfluidic rotating disc for DNA hybridization assays in nanoliter samples proposed by Li et al [222]. Fluid handling is driven by differential rotation of the disk, allowing flow control without the need to interact with the disk. Parallel processing can be performed on multiple channels.

A microfiltration device for the diagnosis of urogenital schistosomiasis was developed by Xiao Y et al. [228], shown in Figure 3.6. In this device *S. haematobium* eggs are trapped and analysed by using brightfield microscopy. For POC use, it might be possible to design this device such that a smartphone camera can be used.

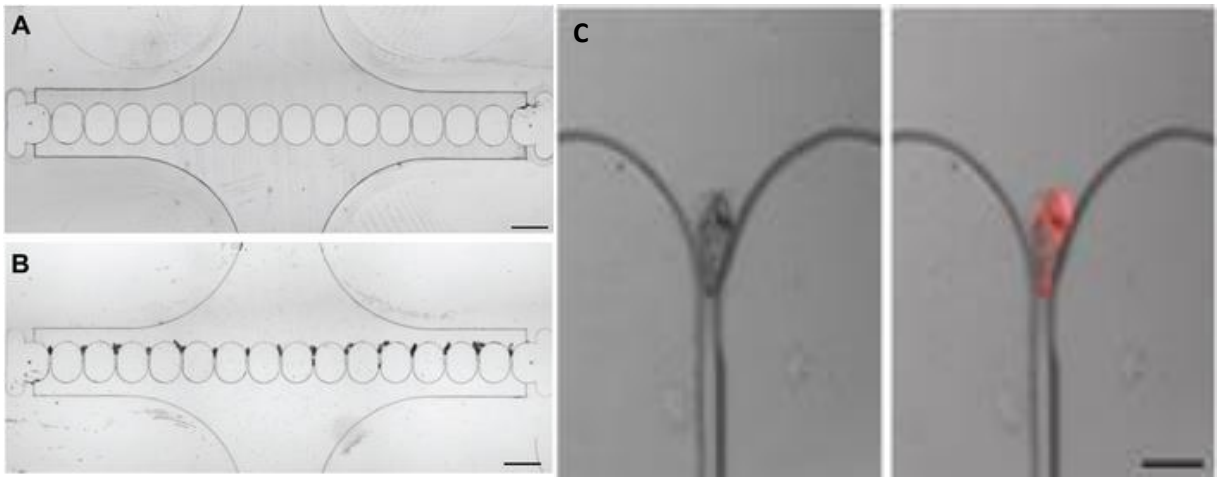

Figure 3.6: Microfluidic device for trapping and fluorescence-based identification of Schistosoma hematobium eggs from urine. A: microfluidic trap. B: trapped schistosoma eggs in brightfield imagine. C: with fluorescence microscopy overlay. Adapted from Xiao Y et al, [229]

Capillary test strips or LF assays can also be considered as fairly simple microfluidic systems and are the most successful POC tests to date. LF devices can be reduced to their bare ingredients - patterned filter paper impregnated with reagents resulting in microfluidic paper-based analytical devices (μPADs). Paper-based techniques allow performing multiple, simultaneous, and independent assays with multiplexed detection. Fast prototyping and production techniques for paper-based diagnostic devices have been proposed [225]. μPADs are inexpensive, easy to use, lightweight to transport, compatible with biological samples and may be handled in remote setting by non-trained personnel.

Ishii [230] developed microfluidic quantitative PCR (qPCR) on environmental samples in a quadruplicate with a dynamic array under specific detection conditions. They found that the quantitative performance of microfluidic qPCR was similar to the conventional qPCR. However, it specifically detected pathogens in stool, demonstrating that this method may be applied to the rapid identification of disease causing-agents for diagnostic purposes. They also quantified multiple targets simultaneously in water samples. Therefore, they concluded that the microfluidic qPCR systems can be applied to the quantification of multiple pathogens in environmental samples. Despite the efforts made in developing microfluidic POC diagnostic devices, microfluidic systems for detection of schistosome infection are developed but still in its infancy due to issues as device sensitivity, multiplex analysis capabilities, assay stability/shelf life, and fabrication cost remain. Standardized evaluation of the performance microfluidic versus other POC technologies in a real-world setting is needed.

Microarrays

Microarrays are usually a glass slide but may also be a micro titre plate or on board of a microfluidic device. Small areas or micro-wells are coated with different probes or biological material for simultaneous detection of multiple targets. In this way, thousands of biomolecular interactions can be probed in parallel [231]. Microarrays can offer multi-component information, dynamic compensation for sample matrix effects, detection of malfunction/deterioration, and improved selectivity through signal pattern analysis. Recent progress has brought microarrays to the forefront of clinical diagnostics and medical research. DNA microarrays appear suited for highly parallel detection and identification of microorganisms from clinical samples [232] and therefore promise more rapid, accurate, and cost-effective detection of pathogens compared to culture techniques or conventional immunoassays.

Genomes of all three major schistosome species have been sequenced [233], and several postgenomic approaches and high throughput methods have been developed to take advantage of this wealth of information [234]. One such approach is a schistosome-specific microarrayed protein, containing 232 unique antigens [235]. Many of these arrayed proteins are novel molecules and the majority is from *S. japonicum*, with the remainder from *S. mansoni*. They can be then probed with antibodies from immune hosts as a powerful new technology for vaccine antigen discovery [236]. Using this approach, McWilliam [237] identified several novel antigens, which may be important targets for vaccine development.

Nano-technology diagnostics

Nanotechnology is based on structures and materials in the nanometre size range. Such nano-size structures and materials differ from microscale structures and materials in various aspects, for example in the very high surface per weight, the occurrence of specific physical effects like particle surface plasmon effects not readily observed in the micro- or macro scale. Gold nanoparticles are stable, have an ability to bind biomolecules and are already used widespread in many different kinds of applications such as imaging and biomedical diagnosis [238]. An example is the amperometric immunosensor for the detection of S. japonicum antigen [239]. This sensor is made of carbon paste coated with a layer of chitosan on which a monolayer of gold nanoparticles was formed. Antibodies against *S. japonicum* antigen were linked to the gold monolayer and used to detect this antigen with a detection limit of 0.06 μg/mL [240]. More recent work is the highly sensitive detection of *S. mansoni* DNA (Santos, 2017), where aminated magnetic particles and gold nanoparticles are immobilized on monolayers of mercaptobenzoic acid. Thiolated DNA probes are immobilized on the surface of the nanoparticles. Evaluation of the surface was performed by using an atomic force microscope and the electrochemical processes (binding of the antigens) were measured by using electrochemical impedance spectroscopy and cyclic voltammetry. *S. mansoni* DNA from cerebrospinal fluid and serum could be detected with a detection limit of 0.685 and 0.781 pg/μL respectively. Such sensors will require further development to render their application suitably simple and robust for application in the field [241].

3.5 Diagnostic tests in the "real world" of field practice

Parasitological methods

In most locations worldwide and in particular in endemic areas, laboratory diagnosis of schistosomiasis is still based on parasite egg detection in faeces or in urine. Direct or indirect microscopy remains in use in POC although being laborious, and of limited sensitivity because of the daily egg count fluctuation. However, negative microscopy does not disprove the possibility of infection in people living in or coming from endemic areas. Results need to be validated by improved diagnostic techniques. Urine microscopy after centrifugation or filtration is required for urogenital schistosomiasis [45]. It presents a good specificity but a low sensitivity consecutive to variable egg release. Nevertheless, in endemic settings, efficiency could reach when urine is collected between 10 am and 2 pm according to the egg excretion pattern [111]. However, during field surveys, urine microscopy presents some difficulties.

Immunoassays

Immune host response against schistosome antigens has been alternative way for diagnosis. For many years several immunodiagnostic techniques have been developed. AbD immunoassays are widely used. However, since CCA and CAA can be detected by substituting other antigens, both processes can indiscriminately be used. Immunoassays based on CAA, SAWA or SEA circulating antibodies detection are already in use. Their validity is usually variable. Immunoassays complete direct microscopy by detection of specific antibodies in serum and in cerebrospinal fluid. AbD is the best tool for field diagnostic activities. However, they usually exhibit a weak specificity. Frequently, reaction with other parasites antigens compromises the specificity. Nevertheless, antigens used in immunoassays have proven crucial test sensitivity and specificity element [51].

Molecular methods

Molecular methods present high performance in use [112, 208]. PCR is highly accurate, highly sensitive and specific for the detection of *S. mansoni*. They also permitted *S. mansoni* increased prevalence estimates, whereas KK assay underestimated them. However, [205] reported discordant results in samples tested by conventional PCR and KK technique, and negative PCR results in K-K positive samples.

RT-PCR was found highly specific and sensitive in *S. japonicum* and *S. haematobium* detection [210]. Compared to microscopy and immunoassays, RT-PCR can be a best marker after chemotherapy [112]. Although owning these qualities, PCR and RT-PCR are cumbersome and costly methods. Nucleic acids extraction stages improvement could perhaps lead to cost reduction [112]. DNA detection development by LAMP technologies promises to be the best alternative [212, 216].

Emerging technologies

The persistent transmission and widespread distribution of schistosome infection call for development of new "gold-standard" diagnostic assays. Identification and detection of infected hosts as well as new diagnostic tests for field application [214] are essential for case-management and disease control. These tests must be more sensitive, more specific, and affordable for point-of-care diagnostic strategies (see also Table 3.3). There are two important challenges: 1) although parasitological tests remain central for schistosomiasis diagnosis, they have proven their accuracy inability, 2) immunoassays and usual molecular techniques have demonstrated their weaknesses. To overcome these shortcomings, resorting to statistical indirect methods to assess the accuracy is frequently required. For example, the use of latent class analysis (LCA) [94, 158, 242] has proven its capability for validation of the sensitivity and specificity of the tests [158].

With the goal of the development of an automated and/or integrated device, microfluidic platforms procure hope. Miniaturization could enable affordable POC diagnostic test for end users in low resource setting [243, 244].

Table 3.3: Criteria/wish list for future diagnostic tests [44, 212, 244]

- ASSURED criteria:
  - **A**ffordable by those at risk of infection (less expensive for patients and for health services);
  - **S**ensitive (few false-negative): able to diagnose light infections (1 EPG for intestinal and 1 egg/10mL for urinary schistosomiasis)
  - **S**pecific (few false-positive);
  - **U**ser-friendly (simple to perform in a few steps with minimal training);
  - **R**obust (Field-adapted: stable in ambient temperature conditions - from 4 to 30ºC) - does not require refrigerated storage) and rapid (results available in 30 minutes)
  - **E**quipment free (less instrumentation, no computer use);
  - **D**eliverable to the end users
- Inexpensive to produce (cost target of less than 1 US$ per test to ensure commercial viability)
- Producing a visual readout (results can be read by naked eyes)
- Using non-invasive samples (urine, stool, saliva, sputum)

3.6 New challenges and uncertainties for more adapted tests in different settings

Clinical scenarios for schistosomiasis test vary widely: For disease control in communities with a very high prevalence, a test with imperfect sensitivity will predictably be associated with a significant number of false negatives, rendering treatment of everybody the most rational approach and implying that individual testing only adds cost without adding benefit and is therefore not required. In contrast, in a low-prevalence situation near the eradication threshold, where infection severity typically is also low, a test with low sensitivity may miss many of those with persistent, but low parasite load, so that the infection cycle is not interrupted and completing eradication becomes infeasible. In this situation, a test with near perfect sensitivity like LAMP would be needed to identify affected individuals, but because most individuals undergoing the test will be negative, such testing may become unaffordably expensive for developing countries. The situation is yet different in travellers from affluent countries, where optimal sensitivity is desired, and event relatively costly tests are economically feasible.

Reliable access to electricity for diagnostic devices, even simple microscopes, is a pervasive problem in developing countries. Fortunately, recent progress in miniaturization, battery technology, low power embedded high performance computing [245] and solar cell technology has led to development of portable, battery driven diagnostic devices for parasitic disease, e.g. portable microscopy [246] and portable molecular diagnostics like LAMP [247] that are suited

for use in very low resource environments. Thus, alternative energies have the capability to benefit developing countries and basic healthcare profoundly.

Despite the impressive advances on the technology front, an integrated approach with practical, adapted, affordable rapid diagnostic tools in combination with suited clinical and epidemiologic testing strategies is still to be defined. Recent clinical observations of lower than expected current cure rates with the mainstay of therapy, praziquantel, particularly when sensitive testing is used for treatment success monitoring, also raise the concern of slow, but progressive drug resistance development. This would add to the challenge for novel tests that not only detect a parasite but also determine its sensitivity to standard drugs treatment.

Table 3.4: Needs of schistosomiasis tests according to different settings

Schistosome infection diagnostic methods	***Needs***
Parasitological	Improved microscopy
Immunoassays	RDTs; Dipsticks
Molecular	Miniaturized LAMP
Emerging	Microfluidic point-of-care devices (mobile phone based)

3.7 Conclusion

Current parasitological methods have well-documented weaknesses. They are often too resource-consuming, laborious or expensive for broad use in endemic areas. In addition, they often do not allow distinction between latent versus recent infection. Immunoassays that have long been perceived as replacement or second option because of their high sensitivity, often fail due to their low specificity. However, combination of several test modality may improve reliability of testing, but this involves increased cost and time consumption. Use of molecular techniques offers a real alternative because they offset the weaknesses of the other methods while enhancing their strengths. Nevertheless, they remain laborious and expensive. The LAMP approach to nucleic acid detection appears to be a valuable platform but needs further development in terms of miniaturization, reduction of carryover contamination risk and develop instrumentation that is field-ready. New microfluidic devices have a significant potential for improving diagnosis based on protein and nucleic acid detection. The integration of multiple test modalities into a robust point of care device that is easy to handle and fulfils the ASSURED

criteria appears technically feasible and when applied to schistosome diagnosis may revolutionize clinical diagnosis and greatly enhance public health efforts in this important disease.

Thus, there remains a significant unmet need for new tests that are highly sensitive, highly specific, adapted to field conditions and allow point-of-care diagnosis, are inexpensive and are suited to contribute either to mass treatment in high prevalence areas, eradication in low-prevalence areas, or individual diagnosis in symptomatic individuals outside such scenarios. The rapid evolution observed in nanotechnologies and microfluidics, as well as in molecular diagnostics, will hopefully render technologic progress also beneficial for the poorest in regions where testing may contribute most to humankind.

4 Schistosomiasis in Eastern Congo: A major neglected healthcare concern in a setting of war and unrest, extreme population poverty, extreme richness in minerals, and minimal infrastructure.

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Short title: Schistosomiasis, a major neglected health care concern in Ituri province, Northeastern Congo

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This working paper is in preparation for a view point article in preparation

4.1 Summary

The great African War, triggered by mineral resources and tribal conflicts, has ravaged regions of Africa, cost millions of lives, destroyed economy, infrastructure and healthcare institutions. Meanwhile, parasitic and infectious diseases have spread like wildfire. Alarmed by informal observations, we studied the prevalence of schistosome infection (a chronic, invasive worm disease) in eastern DR Congo for the first time since decades, explored morbidity, examined demographic, geographic and socioeconomic factors, including 3 153 patients in 59 villages in an area of 65 658 km². Schistosomiasis prevalence was up to 90%, particularly in children, corresponding to millions of undiagnosed and untreated cases in this region alone, spreading far beyond historic hotspots. Predisposing factors included poverty, lack of good sanitation, limited access to clean water, vegetation characteristics, water contact activities, lack of knowledge on disease transmission and prevention. Significant morbidity was found. Profound poverty was contrasted with the richness in minerals exploited; proximity to mining sites was not associated with reduced poverty or reduced disease prevalence. Infrastructure was severely damaged and security was fragile. Past, national healthcare efforts had left little trace, and current Ebola containment activities nearby drain personnel away from basic healthcare. As a result, the common conditions that daily undermine the health of populations are relegated to the background. War and conflicts have led to widespread human suffering; a large population in the region is now affected by a combination of alarmingly high schistosome infection prevalence (on top of malaria and Ebola threats), extreme poverty, and severe infrastructure and security challenges. A holistic rebuilding effort will require politics to plan, facilitate, educate, build up infrastructure and combat corruption. The global mining industry's pledge to invest in local population benefit will be documentable here by demonstrating quantitative progress on this study's data, which also enable non-governmental actors to contribute to humankind where suffering is worst and benefit is highest.

4.2 Background

The Great African War from 1996 to 2006, centring on the east of the Democratic Republic of Congo has cost more lives than the Napoleonic Wars or the Vietnamese War. It is the Africa's deadliest war since the World Second War [248-251]. In one decade alone, between 3.3 to 7.6 million people died with direct and indirect effects of the war [252-255]. The healthcare system in this region largely broke down during that time, e.g. manifested by the massacre of Nyankunde in September 2002 that cost about 1000 lives and destroyed one of the major functioning healthcare centres in the region that provided education for medical professionals, hospital care, and quality pharmacy services [256, 257]. Since the start of the war, epidemiologic data from the region are nearly non-existent. The purpose of this chapter is to briefly present the main results of the surveys we conducted in this region.

Informal personal data and reports from local healthcare workers mentioned a high frequency of *S. mansoni* infection, in addition to the pan-African concerns of malaria, viral disease (HIV, measles, yellow fever, dengue, chikungunya), cholera, soil-transmitted worms, and proximity to recent Ebola outbreaks [258].

As reliable epidemiologic data are an important prerequisite for future poverty-disease related healthcare interventions, we aimed at systematically study disease prevalence and morbidity together with socioeconomic and demographic data, enabling the design of effective, targeted and sustainable future activities.

The Ituri province was considered to be particularly relevant and interesting because health-related data from this area in the core of the Great African War are particularly sparse [13]. In addition, the current time is a transition period for the region that includes hope for progress in peace and security and at the same time rapid expansion of artisanal and in particular industrial mineral mining [250, 259, 260]. The current transition period may thus be critical for implementation of means towards a stable society [261] with aspects ranging from education, security, healthcare and associated economic recovery.

Schistosomiasis occupies an important place on the list of neglected tropical diseases (NTDs) of the World Health Organization [262]. In 2016, there were approximately 221 million cases of

infection worldwide, over 90% of them in sub-Saharan Africa [40]. As for the most of vector borne diseases, schistosomiasis spreads rapidly where climatic and environmental conditions are suitable to the fresh-water snails, the intermediate hosts. Poverty, the lack of sanitation and a limited access to safe water contribute greatly in its transmission and will perpetuate the spread of the disease in the community. Control measures are therefore crucial to break this cycle. The fight against schistosomiasis is based on the following interventions: preventive chemotherapy with praziquantel, control of snails, and water supply, hygiene, and sanitation (WASH). However, because of national health policy and the lack of infrastructures or because of wars and multifaceted conflicts, these strategies may be inadequate, especially in some needy areas, leaving the most vulnerable unreached [39, 263]. This is the case of Ituri in the DRC.

4.3 Socioeconomic, geographic and security context

Ituri province is about 65 658 km², located at the north-eastern DRC. According to the Food and Agricultural Organization (FAO) data, the Ituri province is situated at 0.4 to 0.45 normalized difference vegetation index (NDVI) eco-climatic zones [264]. The province has a population of about 5.282 million people (no census was done in DRC since 1984!) and is divided into five territories and 36 health districts. The Ituri province, a territory of about two times Switzerland presents a strong geographic and ethnic variability. The highest point of the Blue Mountains chain culminates at about 2400 m. The north-eastern of the province is covered by a dense grassy savannah whereas the south-western by the equatorial forest. It is a densely irrigated land. The region is inhabited by various tribes of Sudanese, Nilotic, Bantu, Nilo-Hamite, and Pygmy ethnic background (Figure 4.1).

The population engages in both subsistence and commercial (coffee and cocoa) agriculture. Those living near the Lake Albert, mainly those of the Alur tribe, practice fishing. The main livestock owners are from the Hema tribe. Those living in the forest harvest wood and coal while large companies produce large quantities of wood logs for export (Figure 4.2).

Despite of having exceptional richness in minerals, oil, large forests, a good soil for agriculture, the population lives in a desperate situation. Extreme poverty of significant segments of the

population is sometimes reported but solid socioeconomic data are missing. The healthcare budget of DR Congo was reported to be < 1 billion USD for a population of 80 million people (~12 USD/person/year) but some believe that effective funding reaching peripheral areas like eastern Congo is less than that [265].

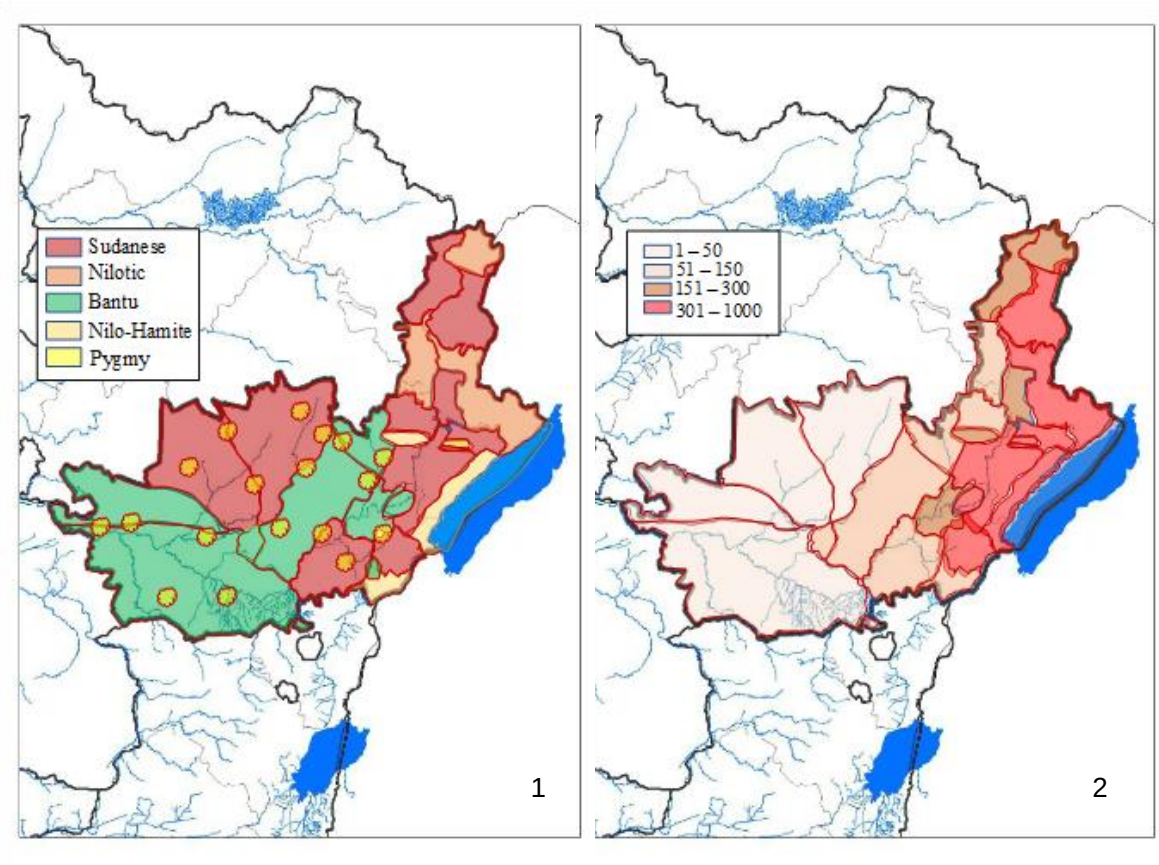

Figure 4.1: Ituri population composition and density: 1) Ethnic groups 2) Density – inhabitants/km²

Economically, the region is one of the richest in sources of minerals in the world, yielding gold, diamonds, coltan, cobalt, copper, and tin in large amounts [266, 267]. Minerals are mined by "artisanal miners", under supervision of the Ministry of Mining, and by large industrial mining companies working within contracts supervised by the country presidency. However, some external interferences were of overwhelming burden to artisanal miners [268, 269]. Industrial mining is highly automated, requiring few local workers. Industrial mining is reported to be highly profitable (Figures 5.3) but data broken down by country or region are difficult to find. For gold

alone, the annual industrial production of the major mining site in the region is 21 tonnes. According to one presentation document, between 2010 and 2018, the main mining company in the region has paid $2.54 billion in the form of taxes, permits, infrastructure, salaries and payments to local suppliers [270]. It is also estimated that between 300 and 600 million US dollars illegally cross the borders of DRC each year [255]. However, a major section of the population is still living in a high level of poverty [271, 272].

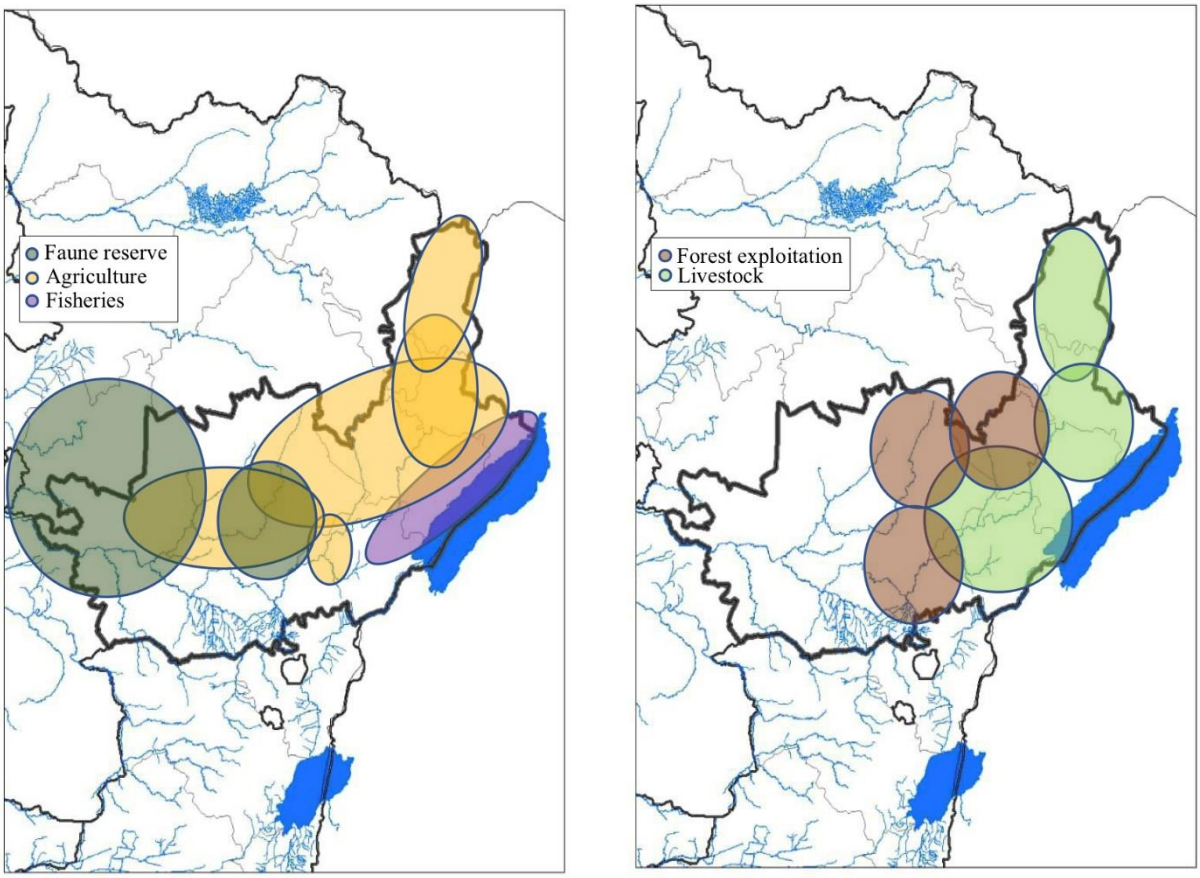

Figure 4.2: Main activities: Faun reserve, agriculture, fisheries, forest exploitation, and livestock

Infrastructure is minimal: the main road ("trans-African") is shown in Figure 1.3. There were more roads in the colonial era than today. The only stretch of 5 km long asphalt road connects the airport used by international peacekeeping forces to the city of Bunia. Mining companies report investments in infrastructure, mainly the access road from the country's border to the mine and

hydroelectric power stations providing the necessary electrical power. Indeed, the best laterite road of about 150 km is the one that connects the Ugandan border to the big gold mine of Kibali in Watsa (Figure 1.4). A lot of unemployed people are mining gold and diamond alongside the large mining companies in the region (Fig 4.3).

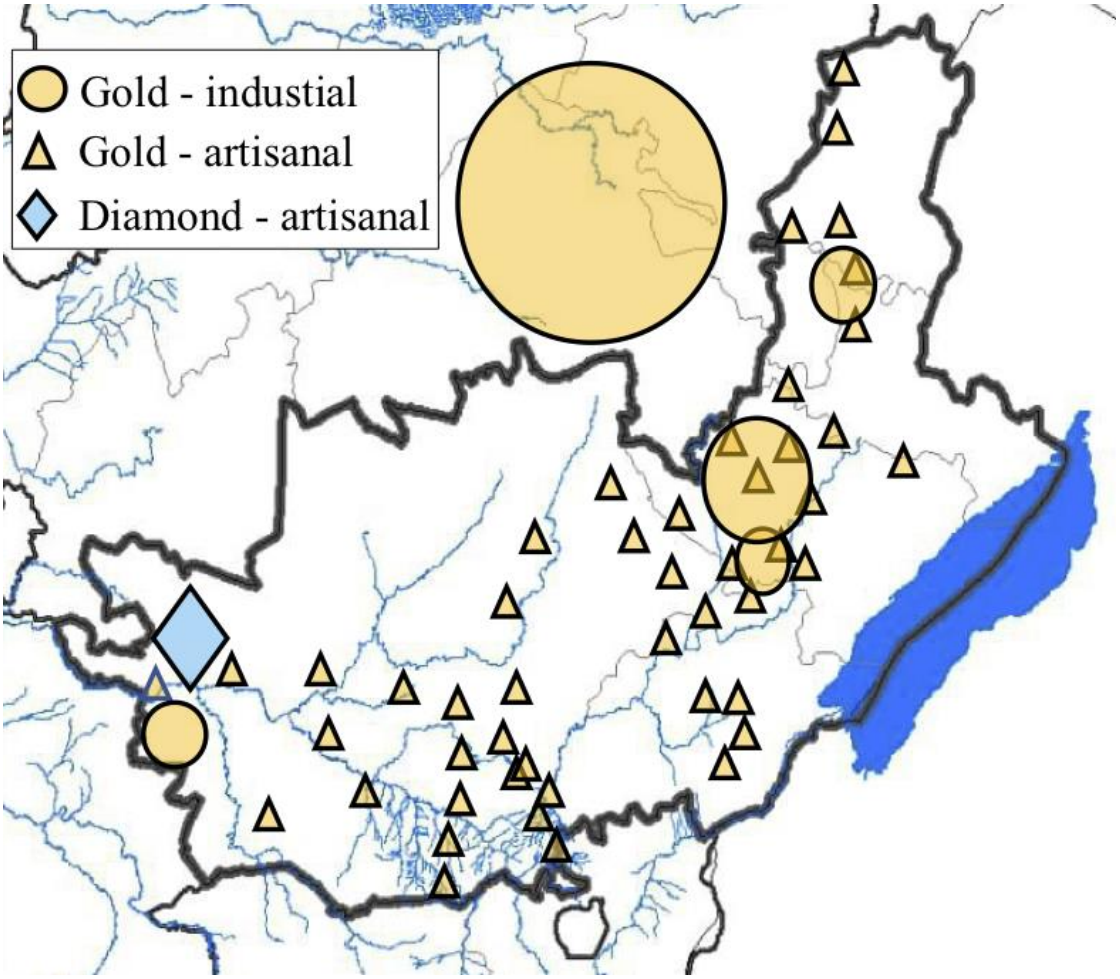

Figure 4.3: Mining both artisanal and industrial

Ituri experienced the throes of Congo's first and second major wars (Figures 4.4). Since then, it continues to suffer the consequences. Although security concerns have improved since the war has subsided various concerns have persisted in the region: roadblocks at different locations are manned during certain times by residents claiming that local resources are extracted with little benefit to the local population. Many armed groups continue to be active in the province fighting for the control of minerals and land. This makes the work environment unsafe. Insecurity remains ubiquitous in the province.

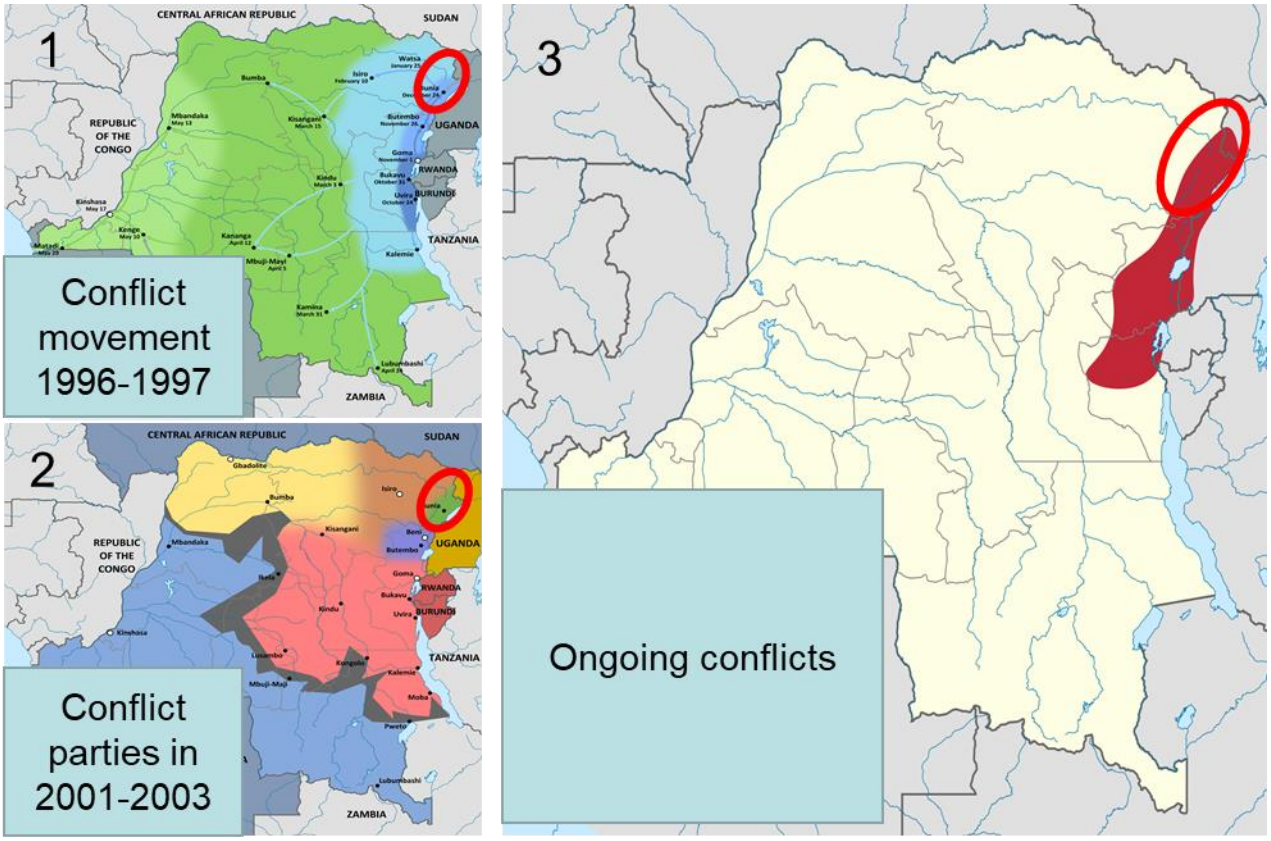

Figure 4.4: Ituri province in midst of the first Great African war from [273]) By Don-kun, Uwe Dedering - Own works, derivates of File:Democratic Republic of the Congo location map.svg, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=30192382; (1) https://commons.wikimedia.org/wiki/File:First_Congo_War_map_en.png; (2) from [274]) https://commons.wikimedia.org/wiki/File:Second_Congo_War_map_en.png; (3) https://commons.wikimedia.org/wiki/File:East_Congo_conflict_map.svg .

Population displacements occur due to security scares (Figure 1.5). People are afraid to leave their homes during the night due to security issues. The territory of Djugu is by far the most disturbed by these repetitive atrocities. Thus, there is massive population displacement of the villages towards the city of Bunia, and towards the territory of Mambasa, or towards the neighbouring Uganda, (Figure 1.5). Beyond all this, Ituri must also deal with rebellions in the neighbouring province of North Kivu and the ongoing Ebola epidemic (Figure 1.6). Also, through manipulation, harassment, and/or extortion, armed persons not otherwise identified would exploit people at artisanal mining sites, claiming that their wages are not being paid to them [266, 268, 269, 275].

4.4 Methods

Two large cross-sectional surveys on schistosomiasis targeting 14 of the 36 health districts of Ituri province were performed. An adage says that "well-ordered charity begins with oneself". In fact, I was born and raised in Ituri. Like all the children in this province, I experienced suffering from parasites such as roundworms, hookworms, filaria, and schistosomiasis. However, the most important thing is not to experiment and to tell one's individual story, but to make sure that never again can it touch the next generation. In 2015, 7 schools were investigated as an exploratory step. In 2016, 12 health districts covering a representative part of the region were randomly selected and investigated. People living in 51 different villages were examined. In 2017, we conducted an in-depth survey on prevalence and morbidity in 13 villages. The DRC is a country of great economic disparities, social inequalities and extreme poverty. The most important health and social indicators include, among others, endemic malnutrition and high infant mortality.
Thus, everybody (aged ≥1 year) living in the visited villages were included in the study. There were no exclusion criteria.

Ethical considerations

Ethical approvals were obtained from Ethik Kommission Nordwest Schweiz [EKNZ]; reference no. UBE-15/78, Switzerland, University of Kisangani Ethical Committee, and Nyankunde Medical Centre High School, DRC. Literate adult participants signed the informed consent. The illiterate put their thumbprint and parents and/or legal tutors gave their assent for individuals aged less than 18 years. The protocols are summarized in the appendix.

Procedures

Between June 15th, and September 15th, 2016, our team which included two nurses, four laboratory technicians, one medical doctor and the team leader screened volunteers aged 1 year or older. Two standardised forms including village form and individual form were filled by the team members. In the village form, information about geographical coordinates, demography, the closest source and type of the water source, the main body of water, and the nearest health facility type and

distance were collected. On the individual form, personal, anthropometric, and parasitological data were completed following WHO guidelines [66].

Between July 1, and September 30, 2017, trained interviewers administered two standardised questionnaire including household questionnaire, and individual questionnaire. In the household questionnaire, information about geographical coordinates, house building materials, distance from the nearby body of water, annual financial income, livestock, household equipment and goods, access to safe water, presence and quality of the latrine, the overall household sanitation, and the most important problems of the village were collected. In the individual questionnaire, information concerning social, demographic and anthropometric characteristics, education, occupation, religion, consumption of water, length of the sojourn in the village, shoe wearing, and knowledge, attitude, and practices related to schistosomiasis were taken. Also, familial and personal history of schistosomiasis were recorded.

Each participant was invited to undergoing clinical and ultrasound examinations at the nearest health facility. During the survey sessions, our research team provided health education to the participants on schistosomiasis and soil-transmitted helminths (STH).

Stool, urine examinations.

In 2016, every participant was asked to provide one stool sample of approximately 5 grams in an appropriate container. However, in 2017, they were asked to provide every day one stool sample for five days. For each stool sample, two Kato-Katz thick smears of about 41.7 mg were made [79]. After microscopy, the mean number of the two slides was used as the daily result. To determine the worm load of the positive sample, the mean egg counts were multiplied by 24 for obtaining eggs per gram (EPG) of stool. Then, the intensity of infections was graded as light (1-99 EPG), moderate (100-399 EPG) or heavy (≥400 EPG). On the fifth day, a 60 to 100 ml of urine sample was required from the participants who provided at least one stool sample [66]. Urine point-of-care circulating cathodic antigen (POC-CCA) lateral flow test was then performed using and following manufacturer recommendations [144]. The CCA, one of the major antigens regurgitated by the schistosomes, is secreted in urine. Briefly, one drop of midstream urine specimen is transferred to the circular well of the test cassette (Figure 2.7). The result of each cassette was read exactly after 20 minutes. The control line must turn from blue to pink. Any line in the test area was considered positive. A positive result was showed by two lines and a negative result by a single line. The tests

were repeated if the results were invalid (when the control line stays blue and/or when the test line appears with no control line). Both Kato-Katz and POC-CCA tests were performed by trained laboratory technicians. At least 5% of Kato-Katz slides were checked by the team leader.
Clinical examinations.

Clinical examinations including anamnesis, palpation and percussion were performed before undergoing ultrasound examinations. Every patient was clinically examined by the same clinical team comprising of one physician, one nurse and two assistant nurses. Abdominal palpations and percussions were performed as to a protocol.

Ultrasound examination

Ultrasound examinations were performed by a trained physician assisted by an experienced nurse. We used a portable U-Lite Sono-Scanner (Paris, France) for this purpose. Patients were asked to fast before the examination and invited to come to the village health centre. However, those with advanced schistosomiasis were offered transportation from and back to their home. All the patients were examined in a supine position. The ultrasound examinations were done following the World Health Organization guidelines [55], with the patients in supine position.

Statistics

Our surveys were based on multi-stage sampling. In total, we included 14 of the 36 health districts of Ituri province (see Figure 2.4). In each selected health district, except one, at least two villages were randomly selected. As there was no recent publication, we assumed that the prevalence was 50.0%. We applied a design effect of 1.5 to account for the random sampling design. Thus, we calculated the sample size using EpiInfo 7.2.1 software (EpiInfo™). The overall sample sizes were 2312 in 2016, and 1045 in 2017. After exclusion of participants who did not provide any stool, nor urine sample, 2131 individuals in 2016 and 1022 in 2017 were included in the final analyses. They were distributed in eight age groups: 1 – 4, 5 – 9, 10 – 14, 15 – 19, 20 – 29, 30 – 39, 40 – 49, and ≥50 years. For both 2016 and 2017 data, descriptive statistics including means, proportions, and ratios were performed. Arithmetic and geometric means of parasites' egg count (EPG) were calculated. A 95% confidence interval (CI) of the above statistics were established. Then multiple

and two-way entry tables were composed. Graphs of means and/or medians by participant category were also made for some numerical and/or categorical variables. A linear regression model of was performed for some variable for determining whether there is a correlation between them. As to the analysis of risk factors among participants who were examined in 2017, an univariate logistic regression analysis was carried out to 1) associate potential risk factors, 2) as well as demographic factors (age and gender), 3) residence factors (domicile, household, village, health district), 4) environmental factors (proximity to bodies of water), 5) geographic factors (altitude), 6) socioeconomic factors (household yearly income), 7) main activities in water bodies (washing clothes, swimming, fishing, farming, cleaning motorcycles), with *S. mansoni* infection status. Pearson's chi-square (χ^2) test was used to examine the differences between the frequency distribution. Odd ratios (OR) and 95% confidence intervals (CI) to determine whether or not there are associations between categorical variables. Multiple logistic regression analysis was then used to identify the main predictors of *S. mansoni* infection. A p-value <0.05 was considered statistically significant both for the χ^2 and the z-score. Also, using a nonuniform spline interpolation [276], a prevalence value map was reconstructed from sparse dataset constituted of a combination of data of our three studies with those from health services.

Treatment of affected individuals.

All sampled participants received 500 mg of mebendazole and all schistosomiasis positive cases were treated on site with praziquantel, 40 mg/kg of body weight.

Outcomes

Following the WHO guidelines, we determined the ultrasonographic findings. We used WHO reference values to define Kato-Katz in the sensitivity analysis, and the manufacturer recommendations for the CCA interpretations.

4.5 Results

National and provincial socioeconomic indicators

The probability of dying before age 5 is 91 per 1000 live births. Only 75% of school-aged children attend school and 54.5% complete primary school. About 85% of households use wood and / or charcoal as the main source of household energy. Only 17.1% of the population has access to electricity, 42.0% have basic access to drinking water, 12.0% of them having limited access, 36.0% using improved water, and 10.0% using surface water. Concerning hygiene, only 2 to 4% have basic access, 11 to 12% have limited access (no water or soap), and 81.0 to 87.0% have no hygiene facility. Finally, concerning sanitation, only 17.1% of population use improved sanitation facilities (excluding shared), 2.6% have septic tanks and 0.1% with sewer connexion.

A total of 59 villages were visited, shown in Figure 2.3 and roads in Figure 1.4. Note that certain areas are practically inaccessible by either car or airplane. In total, 3 153 individuals were examined. All the categories of the population were represented: the five ethnic groups, 52.1% were female and 47.9 male of different age groups, 79.3% from rural areas and 20.7% of urban. Demographic and socioeconomic information about the study population is found in Figure 4.5.

Of all individuals participating in the study, at least one stool sample was available in 98.6%, at least two samples on two different days were available in 91.2%.

Population characteristics

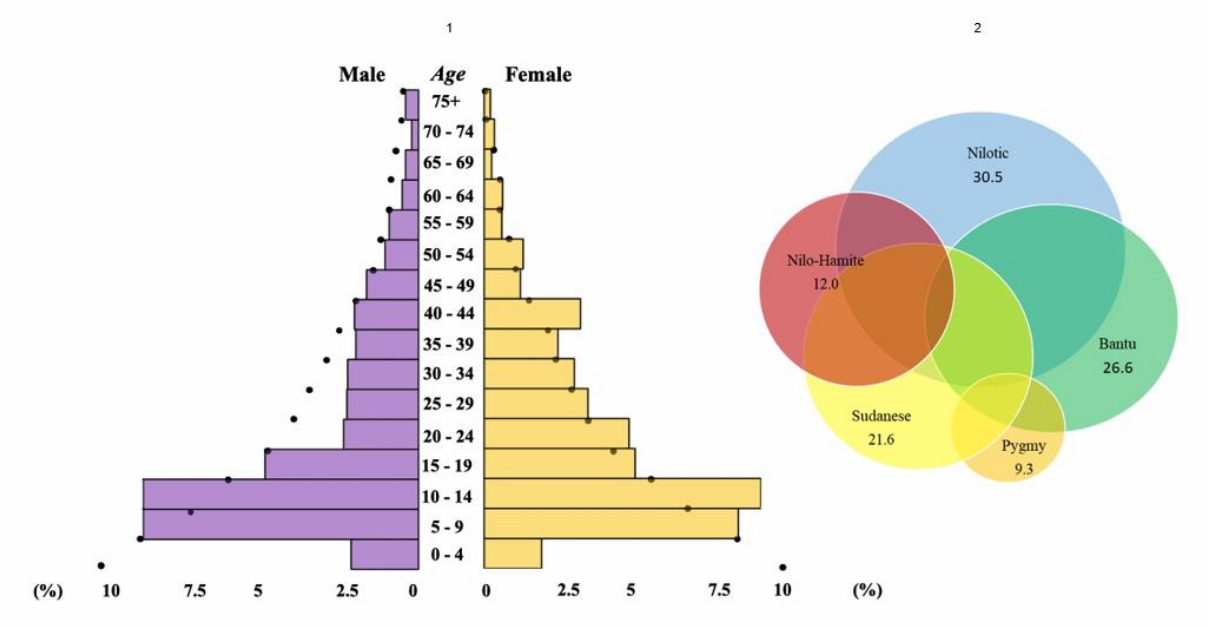

Figure 4.5: Population characteristics: 1) Population structure by age and sex 2) Ethnic composition

Schistosomiasis was prevalent practically throughout the whole region, with prevalence numbers up to 95.0% percent in certain villages (Table 4.1). A prevalence map is shown in Figures 4.8a and 4.8b, and geographic features (altitude, water bodies, forest, roads) are shown in Figures 1.4, and 1.3. Figure 4.1 shows ethnicity and population density.

Socioeconomic factors (Tables 4.1, 4.2 and 4.3) indicated severe poverty, reflected in 64.0% not owning shoes, 17.9% not having latrine and 80.0% using holes as latrines, 16.1% using unimproved tap water and 7.0% using surface water as drinking water, 56.8% not owning transport means, and 91.1% living under the lowest poverty threshold (<1.25 USD/day – PPP). People's life and wellbeing are at stake in this environment. Mining is a key economic factor in the area. Figure 4.3 show the main sites of artisanal and industrial mining.

S. mansoni prevalence was particularly high in villages characterized by their proximity to the water bodies (<500 m); at the lake Albert shore, in low lands (<1000 m altitude), and in forest region; while a few villages at very high geographic elevation (>1900 m altitude) and at the

northern region were found free of schistosomiasis. We found that schistosomiasis prevalence differed significantly among regions, health districts, villages and residence.

Analysis of age and gender documented an important prevalence in all age groups but a particularly high prevalence is found in school-aged children (5 – 9, 10 – 14, and 15 – 19 years), and in young adult groups (20 – 29 years). Schistosomiasis prevalence (by sex and age groups) varied between health districts and villages. Prevalence was not significantly different between men and women for both 1DKK, CCA test and 2DKK+CCA combined tests (Figure 4.7).

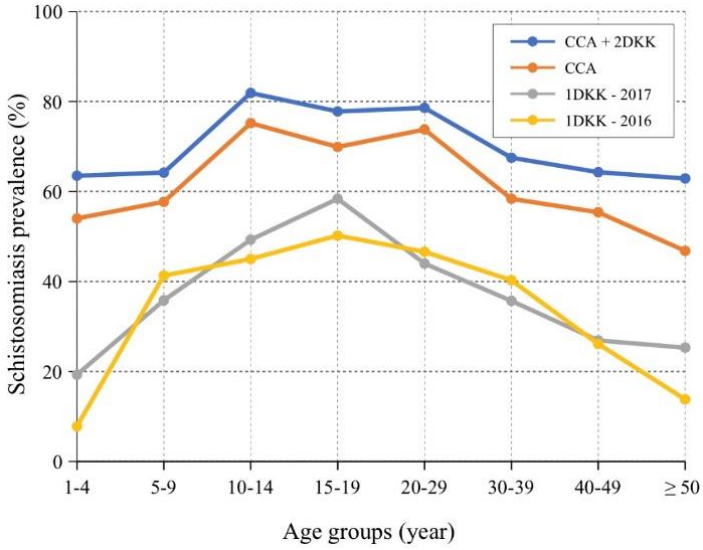

Figure 4.6: Schistosomiasis prevalence both by using one duplicate KK alone in 2016 (grey curve) and in 2017 (yellow curve), and using CCA alone (dark orange curve) or combing two duplicate KK with CCA (blue curve) in 2017.

Table 4.1: Univariate and multivariate analysis of relevant factors associated with schistosomiasis

Risk factor		Prevalence	Univariate		Multivariate	
			OR	p (χ^2)	OR	p (z)
School age	No	69.8				
	Yes	72.7	1.15	0.400		
Gender	Female	70.2				
	Male	72.2	1.10	0.560		
Poverty level	PPP≥1.25$	71.0				
	PPP<1.25$	71.0	1.00	0.997		
Shoes	Yes	65.7				
	No	74.5	1.53	0.011	1.34	0.110
Latrine	Yes	68.2				
	No	84.3	2.49	<0.001	1.88	0.022
Using latrine	Yes	69.4				
	No	78.2	1.58	0.044	1.85	0.014
Water quality	Safe	69.6				
	Unsafe	78.0	1.55	0.059	1.49	0.028
Distance*	≥500 m	62.8				
	<500 m	75.6	1.83	<0.001		
Water contact	Yes	85.3	2.53	0.007	2.09	0.044
	No	69.6				
Bathing**	Yes	85.2	2.41	0.099		
	No	70.5				
Stay duration	≤1 year	60.2				
	>1year	72.5	1.74	0.017	1.65	0.041
Blood in stool	No	69.5				
	Yes	77.3	1.47	0.072		
Diarrhoea	No	69.3				
	Yes	76.6	1.45	0.066	1.37	0.142
Abdomen pain	No	69.0				
	Yes	73.0	1.21	0.240		
Hematemesis	No	70.9				
	Yes	83.3	2.05	0.505		
Splenomegaly	No	69.1				
	Yes	82.2	2.07	0.006	1.69	0.062
Villages	Pekele	96.2	11.43	<0.001		
	Other	69.1				

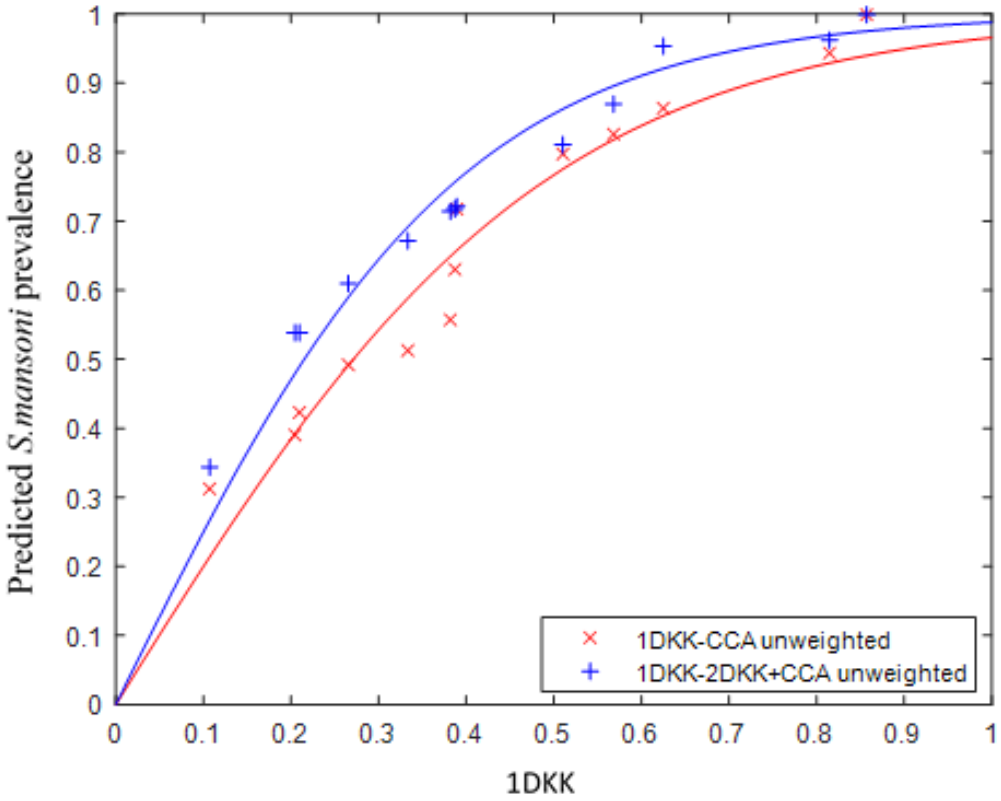

Figure 4.7: Performance of the used tests: Extrapolating village prevalence of schistosomiasis from single-day KK testing to the prevalence measured by more extensive/expensive testing. Blue: relation of prevalence in a village base on single KK to the combination of two duplicate KKs with CCA, using the formula ***prevalence=tanh(2.55*KK)***. Red: relation of prevalence by single-day KK to CCA, using the formula ***prevalence=tanh(2.02*KK)***, where "tanh" is the hyperbolic tangent function (a sigmoid function that crosses (0/0) and approximates 1 for large x).

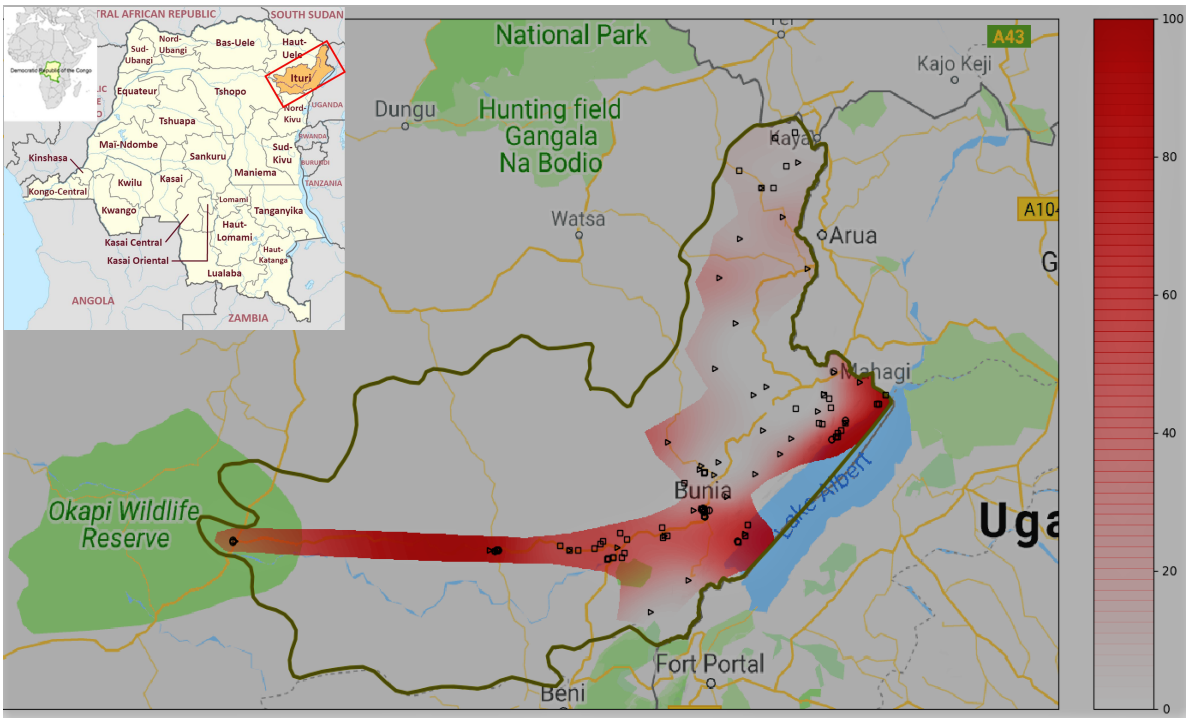

Figure 4.8. Map of Ituri province with estimated *S. mansoni* prevalence. Estimated *S. mansoni* prevalence using non–uniform spline interpolation. Intensity of red shadows is proportional to prevalence levels. Dots indicate studied villages. Areas outside of the red shadow were inaccessible, dense tropical forest and sparsely populated.

We found also that schistosomiasis has evolved since colonial time in the Ituri province. Figures 4.8a and 4.8b shows that the western part of the province that was hypo-endemic has become the main foci of schistosomiasis with prevalence higher than that of the shore of Lake Albert because many people living there are not aboriginals. They moved from the formerly high transmission areas out of fear for their safety or for mining minerals, creating a new schistosomiasis mansoni outbreak.

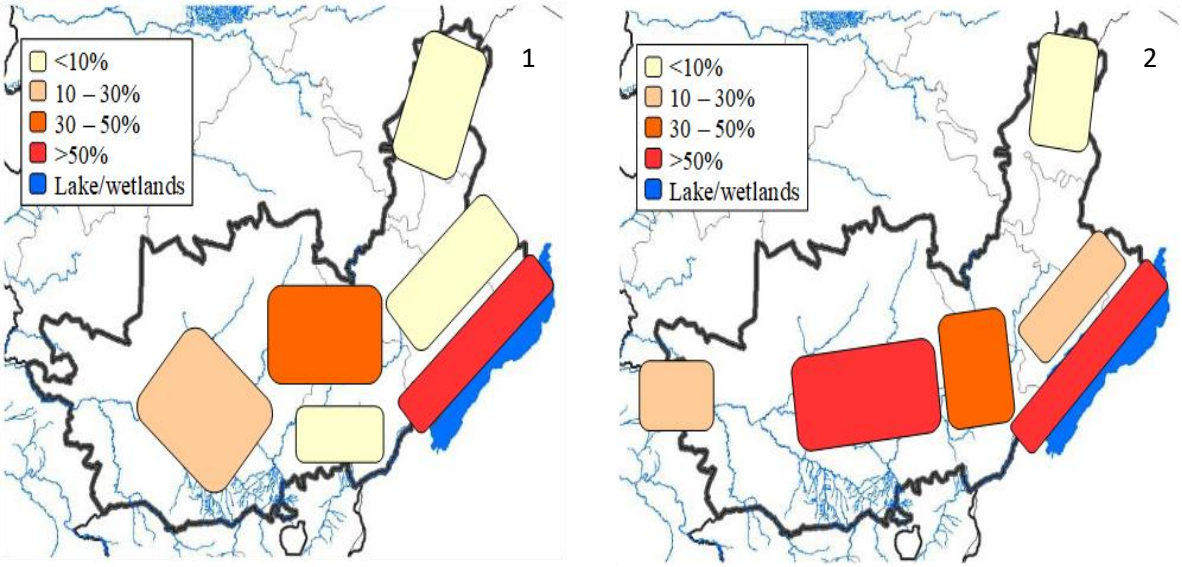

Figure 4.8b: *S. mansoni* prevalence 1) At colonial time (adapted from [118] and 2) Today

Analysis by tribe showed that Alur tribe (Nilotic ethnic group) who live at the lake shore and is the most involved in fishing activities, Lese tribe (Sudanese) and the Pygmies are the most affected by *S. mansoni* infection.

Mineral mining

We also found that prevalence of *S. mansoni* infection and poverty among population were not influenced with mining activities in the area (Figures 4.9, 4.10, 4.11 and 4.12).

Figure 4.9: Industrial mining: problem visible in this image shows irreversible landscape alteration and environment contamination. What can not appear is the large-scale use of lead and mercury that will endanger the health of surrounding population for decades or centuries and that many people are expropriated from their fertile lands and must live in camps.

Images of mining activities in the area: some were taken from Google Earth https://earth.google.com/web/@0.95431441,30.54511868,1290.44726964a,621662.89191239d,35y,30.19560411h,0t,0r and other are live images. What is certain, the mining activities, both artisanal and industrial, will bequeath to future generations only big gaping holes. The environment will be completely destroyed and people will experience worse poverty than the current one.

Figure 4.10: Artisanal mining: if we take into account the existence of thousands of artisanal wells like these, the problems remain the same as for industrial mining; removal of people from their fertile lands, contamination of environment by chemicals such as mercury widely used by artisanal miners, parasites, and microbes. Not to mention the drastically reduced life expectancy of wells' diggers.

Extreme Poverty

Figure 4.11: Poverty in the area: poverty is better illustrated by the quality of housing, the hard and exposing toil of women such as producing palm oil manually, farming, fishing, combined with other household tasks, the clothing of children, lack of shoes, which seriously affect the health of children.

Analysis by activity showed that water contact activities (fishing, farming, washing clothing, dishes, cars, and motorcycles, fetching water, swimming, bathing) and trading presented a high risk of schistosome infection.

The villagers' knowledge about existence of invasive worm disease transmitted by water was very low (77.7% do not know the transmission), and knowledge about disease prevention was also very low (78.0% do not know the prevention).

Multivariate analysis established that environmental factors such as the distance of the village from the main water bodies, the distance of the household from the nearest water body, altitude under 900 m, and the surrounding grass and forest vegetation correlated strongly with schistosomiasis. The age of the participant was found as a protective factor, mainly the lower and the older. However, the group consisted of school-aged children was the most affected. The Sudanese ethnic group, washing clothing in streams, farming activity, and living in a household without latrine appeared to be particularly relevant of risking schistosome infection.

Our results showed that within 91.3.0% of the participants living in clay houses (Figure 4.11), 71.6% were infected and in those 3.6% living in houses made with leaves, 76.0% were infected. Then, among 59.4% of those who did not own shoes, 74.5% were infected. However, 90.5% of those participants from household living with less than 1.25 USD per day, 71.0% were infected. Only 16.1% of people have access to tap water (Table 4.2 and Figures 4.11, 4.12 and 4.13).

Schistosomiasis was not influenced by education. Among 74.4% of the participants of primary school level or low 68.6% were infected, and from 24.6% of secondary school level, 78.1% had schistosomiasis.

Mining and poverty, and mining and *S. mansoni* infection in the area

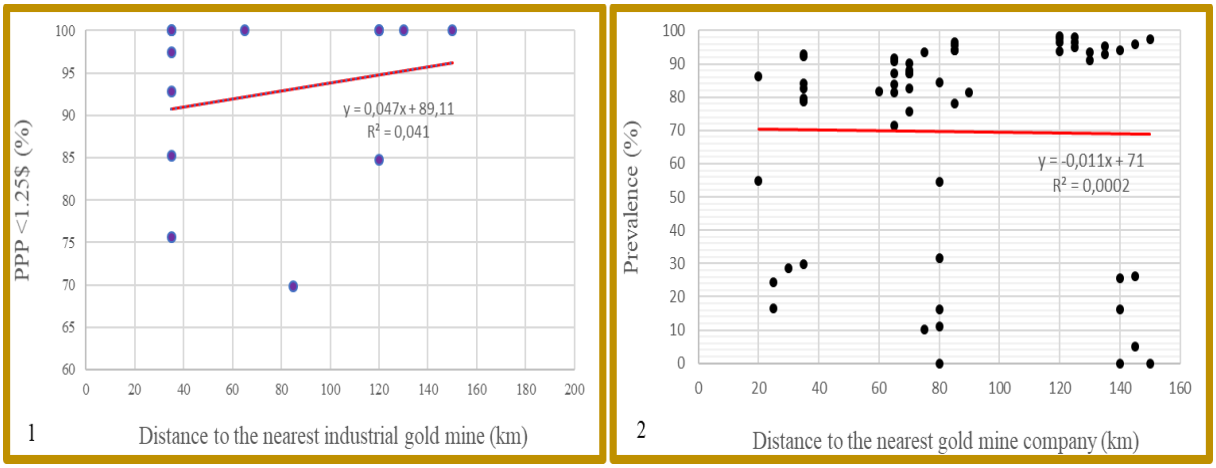

Figure 4.12: 1) Mining and poverty (left): poverty is expressed as percentage of people who have a purchase power parity (PPP) less than 1.25 US$ on the y axis and distance to the nearest industrial gold mine (in km) on the x axis (villages' data, 2017). 2) Mining and *S. mansoni* infection (right) – data of the three studies. There is no indication that industrial mining reduced poverty or *S. mansoni* infection in its surrounding areas.

Water

Figure 4.13: Water issue: whether in town or in the villages, water supply is the greatest issue. The same source of water is often used for drinking, pleasure, washing clothes and dishes, bathing, even washing bicycles, motorcycles, cars, and sometimes as the place where one gets rid of one's urine or excrement.

Table 4.2: Poverty indicators among the surveyed households in Ituri province

Indicators	Category	Proportion (%)
Latrine in the household	None	17.9
	Hole covered with wood	73.1
	Hole with pavement	6.9
	Flush toilet	2.1
House's roof material	Straw or leaves	29.7
	Light galvanized sheets	70.3
House's wall material	Straw or leaves	2.1
	Clay material	93.8
	Cooked bricks	4.1
Drinking water supply	Surface water	7.0
	Natural spring water	54.4
	Wells	22.5
	Tap water	16.1
Means of transport	None	58.6
	Bicycle	15.2
	Motorcycle	24.8
	Car	1.4
Shoes	None	32.7
	Slippers	31.3
	Shoes	36.0
Purchasing power parity (PPP)	<1.25 USD	91.1
	≥1.25 USD	8.9

Table 4.3: Other socioeconomic indicators in both national and provincial levels

Indicators	National	Provincial
Reading capability	61.2%	-
School education (primary)	74.8%	-
Finish primary school	54.5%	-
Life expectancy	49.6 years	-
Living below PPP threshold	87.7%	91.1%
Number of people owning telephone	28.0%	-
Number of people using internet	1.7%	-
Total number of hospitals	527	40
Number of health centres	7868	190
Number of physicians/1000	0.073	0.045
Number of pharmacists/1000	0.016	0.002
Number of dentists/1000	<0.001	<0.001
Number of nurses/1000	0.524	0.237
Number of lab technicians/1000	0.011	0.01

Roads

Figure 4.14: Roads' issue: one of the major issues in Ituri province is that of roads. They are so dilapidated that driving in vehicles becomes sometimes impossible. Often, vehicles get bogged down frequently in muddy waters. Sometimes the road can disappear and the vehicle can suffer serious damage. Many bridges built in colonial times are washed away or broken. Thus, several rivers are crossed by fording, which is very risky. The best and biggest bridge of the province is shown on the middle top of this figure. All these issues cause a great loss of time, which is a challenge to perform planned activities.

Morbidity associated with *S. mansoni* infection

Morbidity (Figures 4.15) assessment showed that among infected people, 52.4%, 24.5%, and 22.1% had respectively abdominal pain, diarrhoea, and blood in the stool within the two weeks. We detected splenomegaly and hepatomegaly by physical examination respectively among 16.9% and 5.4% of infected people. After ultrasound examination, 55.7% and 41.0% presented respectively splenomegaly and hepatomegaly. Also, ultrasound examination of liver portal vein showed that respectively 10.1%, 24.5%, 13.6%, and 1.6% had incentive (IPF), probable (PPF), definite (FPF), and advanced (APF) periportal fibrosis. Only 1.0% of them had an history of hematemesis, and 0.4% presented ascites. Of patients with hematemesis, all were schistosomiasis positive, and of those with identifiable ascites, only 50% were positive. However, both reported a history of treatment for schistosomiasis.

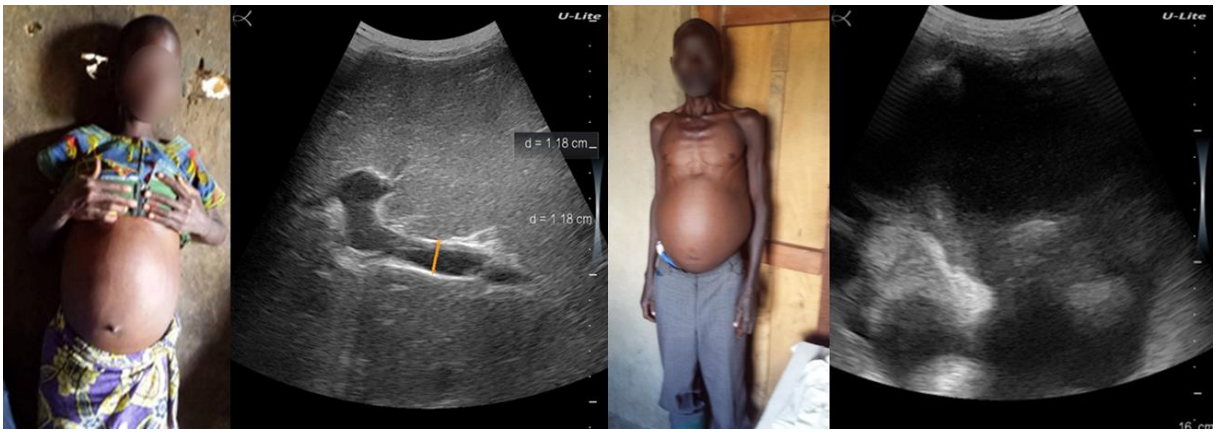

Figure 4.15: Morbidity associated with schistosomiasis: 1) Left: A 22 years old young woman with a large spleen, collateral veins, portal hypertension, and periportal fibrosis; 2) Right: A 51 years old man with liver cirrhosis and generalized ascites in terminal phase of the disease.

4.6 Discussion

This study uncovers an alarmingly high prevalence of the invasive worm disease, schistosomiasis, in one of the poorest populations of the world, suggesting millions of undiagnosed and untreated cases, unfortunately hitting children the most and inducing long-lasting health effects. It documents important morbidity in this war-ravaged population that is related at least in part to schistosomiasis but also due to other prevalent poverty diseases.

It maps schistosomiasis prevalence and morbidity into geographic and demographic variability within the province and this yields important information for designing effective strategies to combat and ultimately eradicate this disease.

Schistosomiasis is a locally eradicable disease: it as has been demonstrated in Japan [31], in large areas of China, Brazil, and the Caribbean, and in some leading African countries such as Egypt and Morocco which are making progress to effectively eradicate the disease [38, 40, 152, 262, 277]. Indeed, this type of success can be achieved if there is national concern and when control programs are well structured and well conducted. Some countries in sub-Saharan Africa have made great progress. Indeed, in 2012, the World Health Assembly adopted the resolution WHA 65.21 [15] calling on governments and health organizations to move from control to elimination of schistosomiasis, and a substantial number of people have been treated [278]. Since then, DR Congo has launched its control program. However, the burden of disease remains unknown [13, 53, 62, 123, 126, 127], this control program is based on data from health service reports or imputed satellite data from neighbouring regions of Africa [13] or from a small portion of population such as school-aged children [279]. However, since schistosomiasis is essentially focal, data from one corner of the country or from other regions may not necessarily be equivalent [135, 280]. This problem is evident in the national program for mass drug administration (MDA) for neglected tropical diseases (NTDs). According to this plan, in communities where prevalence is ≥50.0%, school-aged children and adults receive an annual dose of praziquantel; in communities where the prevalence varies between 10.0-49.0%, only children aged 5 to 14 receive praziquantel (at school) every other year; and where the prevalence is less than 10.0%, praziquantel is given to children aged 5 to 14 every three years. This strategy is the one that is currently applied in the Ituri province since 2016 (MDA/NTDs Ituri). Thus, for the whole province, only the health district of Tchomia (about 114000 inhabitants at the shore of lake Albert) where the prevalence declared by the health services

is higher than 50.0%, theoretically all the children of school age and the adults should be treated annually against schistosomiasis. This seems inprobable. In fact, based on the results of our study, which show that most of the province is hyperendemic (≥50.0%) the entire population or at least all school-aged children and all adults should receive treatment on an annual basis. Also, this program seemed to us theoretical. For, during our visit to the health district of Tchomia (prevalence equals 67.1%) in 2015, 2016 and 2017, no one has told us about the effectiveness of this program. Our results corroborate with Envision who stated that until 2018, no adults actually received treatment in the whole country [132]. Also, in all households visited in 2017, only a few people had heard of praziquantel. Some of them, especially the intellectuals and fishermen, told us that they had bought it at the pharmacy or received it at the district general hospital on the doctor's prescription. In the Angumu health district (north of Tchomia – and 73.0% prevalence), when we visited the households, some people recognized that the praziquantel distribution campaign was about to begin. However, it was on condition that each household should build a latrine. Although it makes sense to recommend the construction of latrines by households, we believe that the praziquantel distribution program should not be conditioned in this way. This is likely to be poorly perceived by the population. In this way, we note that a significant portion of the population of the Ituri province is excluded from the mass distribution program of praziquantel. This is a very dangerous situation as a significant portion of the untreated population will perpetuate the spread of the disease. And all the resources committed to the treatment of only part of the population would be wasteful, annihilating all control efforts.

Thus, adapted to the current situation in eastern Congo, the most realistic activities include burden-of-disease mapping, improved control, interruption of transmission, and elimination of schistosomiasis, while for the current infrastructural, societal and economic situation needs to be improved for successful implementation.

In a country known for its high corruption level, how then will it be possible? Nonetheless, understanding the prevalence distribution and correct estimation of schistosomiasis cases in the province are crucial for determining strategies and setting priorities. Our results showed that schistosomiasis is a health problem of high priority in the Ituri province. It is well known that social context is highly involved in schistosomiasis burden [281]. These findings imply that both governmental and civil society organizations need to identify the best ways to find comprehensive

responses to *S. mansoni* infection. Control of schistosomiasis requires multisectoral interventions [39, 40, 126, 130, 282]. These responses include promoting water, sanitation and hygiene (WASH) [282] activities by providing safe water to the population, raising awareness of behavioural change [39], providing preventive chemotherapy twice a year [38, 155, 156], improving the economic situation of families and households [40], and ensuring access to health services by creating health insurance organizations. This will enable families to be well protected against financial risks and improve their access to appropriate interventions. Health workers need also to be trained in case management. For example, a three-year training period for a nurse or a lab technician in the local college; we found that many families are not able to pay the fees of about 3000 USD. At the same time, many people do not have a job. The major part of the population is not covered by health insurance, as existing insurance companies are expensive and do not respect the commitment clauses. Some efforts would therefore be directed towards increased creativity. Communities should also ensure that their members are aware of schistosomiasis control and that they participate in improving their environment and other control activities. Our results therefore call for the design of a sustainable effort in conjunction with local development organizations and government bodies, and international organizations pursuing the goal of combating and eliminating schistosomiasis in eastern Congo. Indeed, as long as primary education is inaccessible for a large portion of school-aged children (>30.0%) [283, 284], ignorance will continue. Also, as health workers continue to receive a guaranteed minimum wage (currently <5.00 USD per day), there will not be enough motivation for the promotional, preventive and curative activities that fall to them. And corruption is likely to take over. As long as the costs of high-performance diagnostic tests such as CCA are so high (currently >10.00 USD), and as long as the price of praziquantel is as it is offered by most local pharmacies (currently >1.50 USD), curative care for schistosomiasis will remain inaccessible to the majority of the population who are languishing under the weight of extreme poverty.

Paradoxically, the immense riches of the region have cast it into the abyss of a catastrophic war that has also ruined its modest healthcare institutions in the recent past, but these riches may also hold the key to creating the economic basis for a self-sustained society that can provide education, healthcare, security and thus, welfare to their citizens. But this only happen if ways are found how to ensure that the resources of the country lead to the substantial benefit for all – the local residents, the region, the country.

We intend to establish, in collaboration with the population, a control program that will be fully executed by empowering the population itself. This will help to fight against corruption that has always characterized and accompanied for many years the many programs that supposed to intervene for the benefit of the population, but without tangible results. Together, hand in hand, our efforts will succeed.

This responsibility towards the future is in the hands of every citizen and their commitment to work, to self-reliance, to integrity and to social responsibility. But is even more in the hands of the local as well as national governments to show their commitment not to own but to serve their country and to combat corruption at all levels. In this context, the responsibility is no less in the hands of international mining companies, who know that extracting excess profits from a region without substantial and actual participation of the local population in their region's richness will lead unavoidably to future conflicts. And this is by no means the interest of anyone.

5 Epidemiology of *Schistosoma mansoni* infection in Ituri Province, Northeastern Democratic Republic of the Congo

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Short title: Schistosomiasis in Ituri Province in Northeastern Congo

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This article is in revision in the *PLoS Neglected Tropical Diseases journal*

5.1 Abstract

Background

Schistosomiasis, caused by *Schistosoma mansoni*, is of great significance to public health in sub–Saharan Africa. In the Democratic Republic of Congo (DRC), information on the burden of *S. mansoni* infection is scarce, which hinders the implementation of adequate control measures. We assessed the geographical distribution of *S. mansoni* infection across Ituri province in north-eastern DRC and determined the prevailing risk factors.

Methods / Principal Findings

Two province–wide community–based studies were conducted. First, in 2016, a geographical distribution study was carried out in 46 randomly selected villages, covering 12 of the 36 health districts across Ituri. Second, in 2017, an in–depth study was conducted in 12 purposively–selected villages, across six health districts. In each study village, households were randomly selected and members, aged one year and older and present on the survey day, were enrolled. In 2016, one stool sample was collected per participant, while in 2017, several samples were collected per participant. *S. mansoni* eggs were detected using the Kato–Katz technique. The 2017 study also incorporated a point–of–care circulating cathodic *S. mansoni* antigen (POC–CCA) urine test. Household and individual questionnaires were used to collect data on demographic, socioeconomic, environmental, behavioural and knowledge risk factors.

The 2016 study included 2,131 participants, 40.0% of whom had *S. mansoni* infections. Infection prevalence in the villages ranged from 0 to 90.2%. The 2017 study included 707 participants, of whom 73.1% tested positive for *S. mansoni*. Infection prevalence ranged from 52.8 to 95.0 % across the health districts visited. In general, infection prevalence increased from north to south and from west to east. Exposure to the waters of Lake Albert and the villages' altitude above sea level were associated with the distribution.

Both men and women had the same infection risk (odds ratio [OR] 1.2, 95% confidence interval [CI] 0.82–1.76). Infection prevalence and intensity peaked in the age groups between 10 and 29 years. Preschool children were highly infected (62.3%). Key risk factors were poor housing

structure (OR 2.1, 95% CI 1.02–4.35), close proximity to water bodies (OR 1.72, 95% CI 1.1–2.49), long–term residence in a community (OR 1.41, 95% CI 1.11–1.79), lack of latrine in the household (OR 2.00, 95% CI 1.11–3.60), and swimming (OR 2.53, 95% CI 1.20–5.32) and washing (OR 1.75, 95% CI 1.10–2.78) in local water bodies. A family history of schistosomiasis (OR 0.52, 95%, 95% CI 0.29–0.94) and knowledge of praziquantel treatment (OR 0.33, 95% CI 0.16–0.69) were protective risk factors, while prevention knowledge (OR 2.35, 95% CI 1.36–4.08) was associated with increased infection risk.

Conclusions/Significance: Our results confirm high endemicity of *S. mansoni* in Ituri province, DRC. Both the prevalence and intensity of infection, and its relationship with the prevailing socioeconomic, environmental, and behavioural risk factors indicate intense exposure and alarming transmission levels. The study findings warrant control interventions that pay particular attention to high–risk communities and population groups, including preschool children.

Keywords: *Schistosoma mansoni;* Ituri province; Democratic Republic of Congo; infection prevalence; infection intensity; risk factors; geographical distribution.

5.2 Author Summary

Intestinal schistosomiasis threatens many people in the tropical world, particularly those in Sub–Saharan Africa. Information on schistosomiasis in the Democratic Republic of Congo (DRC) is very scarce, which is a major barrier to planning and implementing efficient control programmes.

We conducted two community–based studies with the objective of assessing the geographical distribution of *S. mansoni* infection across the Ituri province in north-eastern DRC and determining the prevailing risk factors. In 2016, a geographical distribution study was carried out in 46 randomly selected villages across the province. In 2017, an in–depth study was conducted in 12 purposively selected villages. In both studies, households were randomly selected and members, aged one year and older and present on the survey day, were enrolled. In 2016, one stool sample was examined per participant, whereas several stool samples were examined for each participant in 2017. *S. mansoni* eggs were detected using the Kato–Katz technique. The 2017 study also included a point–of–care circulating cathodic *S. mansoni* antigen (POC–CCA) urine test. Household and individual questionnaires were used to collect data on demographic, socioeconomic, environmental, behavioural and knowledge risk factors. The 2016 study included 2,131 participants, 40.0% of whom had *S. mansoni* infections. The 2017 study included 707 participants, of whom 73.1% tested positive for *S. mansoni*. In general, infection prevalence increased from north to south and from west to east. Exposure to the waters of Lake Albert and villages' altitude above sea level were main drivers of the distribution. A risk factor analysis revealed that both men and women had the same infection risk. Infection prevalence and intensity peaked in the age groups between 10 and 29 years. Preschool children were highly infected (62.3%). We identified the main risk factors to be poor housing structure, proximity to water bodies, long–term residence in a community, lack of latrine in the household, and swimming and washing in local water bodies. A family history of schistosomiasis and knowledge of praziquantel treatment were protective risk factors, while prevention knowledge was associated with increased infection risk. Our results confirm that *S. mansoni* is highly endemic in Ituri province, DRC. Both infection prevalence and intensity, and its relationship with the prevailing socioeconomic, environmental, and behavioural risk factors indicate intense exposure and alarming transmission. Control interventions are warranted and should pay attention to high–risk communities and population groups, including preschool children.

5.3 Introduction

Schistosomiasis is widespread in sub-Saharan Africa (SSA) where it constitutes major health problem. Human infection is caused by six species of a trematode of the genus *Schistosoma*. Schistosomiasis is transmitted by the eggs of the parasite present in stools and/or urine of infected people, which contaminate the nearby water, then freshwater snails from which infective larvae are released and penetrate the skin of people during contact with infested water. The disease is multifactorial: its transmission is very focal and lies on environmental, parasitic, vector and host factors, including socio-economic and behavioural factors amongst them the lack of proper and adequate sanitation, safe water, and hygiene (WASH). It represents a major cause of global disability, morbidity, and mortality in the affected regions [280, 285]. Recent estimates suggest that nearly 800 million people are at risk for schistosomiasis, while 240 million are infected worldwide [286]. More than 90% of all infected people live in sub–Saharan Africa [40]. The main control strategy is the population-based preventive chemotherapy (PCT) using praziquantel (PZQ) [287].

In SSA, four species of *Schistosoma* including *Schistosoma mansoni* which causes intestinal schistosomiasis, *S. haematobium,* the agent of genitourinary schistosomiasis, *S. intercalatum* and *S. guineensis* [288].

Democratic Republic of Congo (DRC) has the third highest reported number of cases in SSA (15 million), just after Nigeria (29 million) and United Republic of Tanzania (19 million) [7]. In DRC, three species of *Schistosoma* are reported to be present: *S. mansoni*, *S. haematobium*, and *S. intercalatum*. *S. mansoni* is abundant in the eastern region of the country along the shores of the great lakes, and in the western region, whereas *S. haematobium* is present in the central and south-eastern regions, and *S. intercalatum* in the central-northern regions of the country [123].

Intestinal schistosomiasis is one of the major neglected tropical and poverty–related diseases. *S. mansoni* thrives in tropical and sub–tropical regions with poor sanitation conditions [40, 280, 289]. *S. mansoni* alone threatens about 393 million people in Africa, the Middle East, Brazil, Venezuela, Suriname and the Caribbean, and about 54 million are infected [35]. Children bear the highest burden of infection because of their inadequate hygiene and frequent contact with infected waters [290].

In the DRC, the current burden of this neglected tropical disease (NTD) is unknown [13]. The DRC suffers from a recent history of war and ongoing tribal and armed conflicts, the results of which have led to economic deterioration, severe poverty, and badly functioning health services. Data from the available large surveys are more than twenty–years old [62, 123]. Existing publications reporting on schistosome infection focus on different topics [291] or mostly describe epidemiological studies carried out in a few areas of the country [123], such as Kinshasa [53, 128, 292, 293], the capital city; and in the provinces of Kongo Central [68, 294, 295], Bandundu [296] Kwilu [126, 127], Kasaï,Central, Kasaï Oriental [62], Maniema [297-302], South–Kivu [303], Katanga [304, 305] and Haut-Uele province [306].

Publications relating to the north-eastern provinces are scarce [298, 303, 306] or date back to the colonial period [123, 302]. In 2012, the Ministry of Health (MOH) launched a national survey of NTDs and a new strategic plan was elaborated in 2016 [132, 134]. Although a national plan against NTDs, including schistosomiasis, has been implemented by the Ministry of Health, the intervention strategy is based on outdated prevalence data in many regions. Ituri province in north-eastern DRC is one such case, in which the strategy applied is for moderate–risk settings (10 – 49%), yet it appears to be a high–risk area. In this situation, the large number of infected and untreated people will contribute to perpetuating the transmission as long as treatment coverage remains inadequate [38]. Therefore, it is urgent to update our knowledge regarding the distribution of the intestinal schistosomiasis to undertake appropriate intervention and assessment.

The aim of this work was (i) to assess the infection prevalence and intensity of *S. mansoni* and (ii) to describe the main epidemiological features by identifying key risk factors of *S. mansoni* infection in Ituri province, north-eastern DRC.

5.4 Materials and Methods

Ethics statement

This study was approved by the Swiss Ethical Commission (Ref. No. UBE–15/78) and by the University of Kisangani's Research Ethical Commission, (Ref No: CER/003/GEAK/2016). Research authorization was granted by the Nyankunde Higher Institute of Medical Techniques (Ref No 70/ISTM–N/SGAC/2017), Bunia, DRC. Permission for field work was obtained from Ituri Provincial Health Division (Ref. 054/433/DPS/IT/06/2016 and Ref. 054/472/DPS/IT/06/2017) and from all relevant health districts. Prior to enrolment, the study objectives and procedures were explained to the participant in the local language. All participant questions were answered. Written informed consent was obtained from all study participants aged 15 years and above. Parents or legal guardians signed consent forms for participants aged 1–14 years. All participants diagnosed with *S. mansoni* infection were treated with praziquantel (40mg/kg) [141]. All participants received Mebendazole (500mg, single dose, Vermox®) for general deworming, in accordance with the DRC national deworming guidelines.

Study area

Ituri province is situated in north-eastern DRC and has a surface area of 65,658 km² (Figure 5.1). It is divided into counties – territories – (Aru, Mahagi, Djugu, Irumu, and Mambasa) and 36 health districts (See Figures S5.2a and S5.2b). Ituri province is heavily irrigated by natural water streams. It is characterized by a great geographical and demographic variability (Figure S5.1). Aru territory, in the north, is a plateau area of about 1,100 m above sea level and covered with a half-wooded and half-grassy savannah and forest galleries. Next is Mahagi territory, showing almost the same vegetation characteristics as that of Aru. However, the altitude peaks at around 1,800 m followed by a steep slope which descends to lake Albert to the east. Djugu is the high hill (up to 2,300 m above sea level in the Blue Mountains chain) territory of the Ituri province. The largest area is covered with grassy savannah and few forest galleries in the west. The mountains slope steeply towards Lake Albert to the east. Irumu territory shows the same characteristics than Djugu in the east whereas it is covered with dense forest in the west. Mambasa territory is a lowland region totally covered with equatorial dense forest. The health districts are distributed among territories according to the population density. Thus, different district health centres and communities are in

different ecological settings. In all the health districts, curative and preventive activities such as vaccination, infants' and prenatal clinics, deliveries, and human immunodeficiency virus (HIV) testing are currently organized. Some health districts do tuberculosis and leprosy care and control activities. Laboratory diagnostic activities for current diseases such as malaria and soil-transmitted helminths are available in all the district general hospitals. However, many health centres lack diagnostic tools and/or personnel. Praziquantel is available in all the health districts bordering the lake. Neglected tropical diseases (NTDs) drugs are sometimes provided in some health districts by the provincial branch of the national control programme. About 5.3 to 9.0 [307] million people of Sudanese, Nilotic, Bantu, Nilo–Hamite, and Pygmy ethnicities live in Ituri province (Figure S5.3). About 91% of the population live in 45.0% of the northern and eastern areas of the province. As unemployment is a major scourge in DRC, most of the population, both in rural and urban areas, is mainly engaged in subsistence farming. Some, however, raise small and large livestock. Young people are involved in the artisanal exploitation of minerals such as gold, diamonds and coltan. Cattle breeding is mainly practiced in Djugu, Irumu, Mahagi and Aru territories. Fishing is the main activity of people living by the lake and timber harvesting for commercial and domestic purposes is widely practised in the forested areas of the province, particularly in the Mambasa territory. These occupations coupled with the general lack of safe water put the population in frequent contact with contaminated waters of streams and lake which increases the exposure to water-transmitted parasites.

For more than two decades, Ituri province has been subject to war, turmoil, and social conflict. The socioeconomic situation in Ituri province is challenging, with a high degree poverty and precarity. The DRC ranked 176/188 in the Human Development Index in 2017 [308]. In 2011, a Water and Sanitation Program (WSP) strategic overview estimated that 50 million Congolese (75.0%) did not have access to safe water, while approximately 80–90% did not have access to improved sanitation [309]. Likewise, the UNICEF/WHO [310] 2017 database showed that in 2015, 84% of DRC's rural population had no hygiene facility, 45.3% had unimproved sanitation, 10.2% resorted to open defecation, 53% used unimproved water sources, and 16.0% used surface water. According to data from the Food and Agricultural Organization (FAO), Ituri province measures 0.4 to 0.45 on the normalized difference vegetation index (NDVI) [264] (Figure S5.1). Since 2018, Ituri province is experiencing its first Ebola epidemic and as the COVID-19 pandemic emerges currently as a huge

global problem, this will make the situation even more complicated, not to mention all the people that will get ill and die.

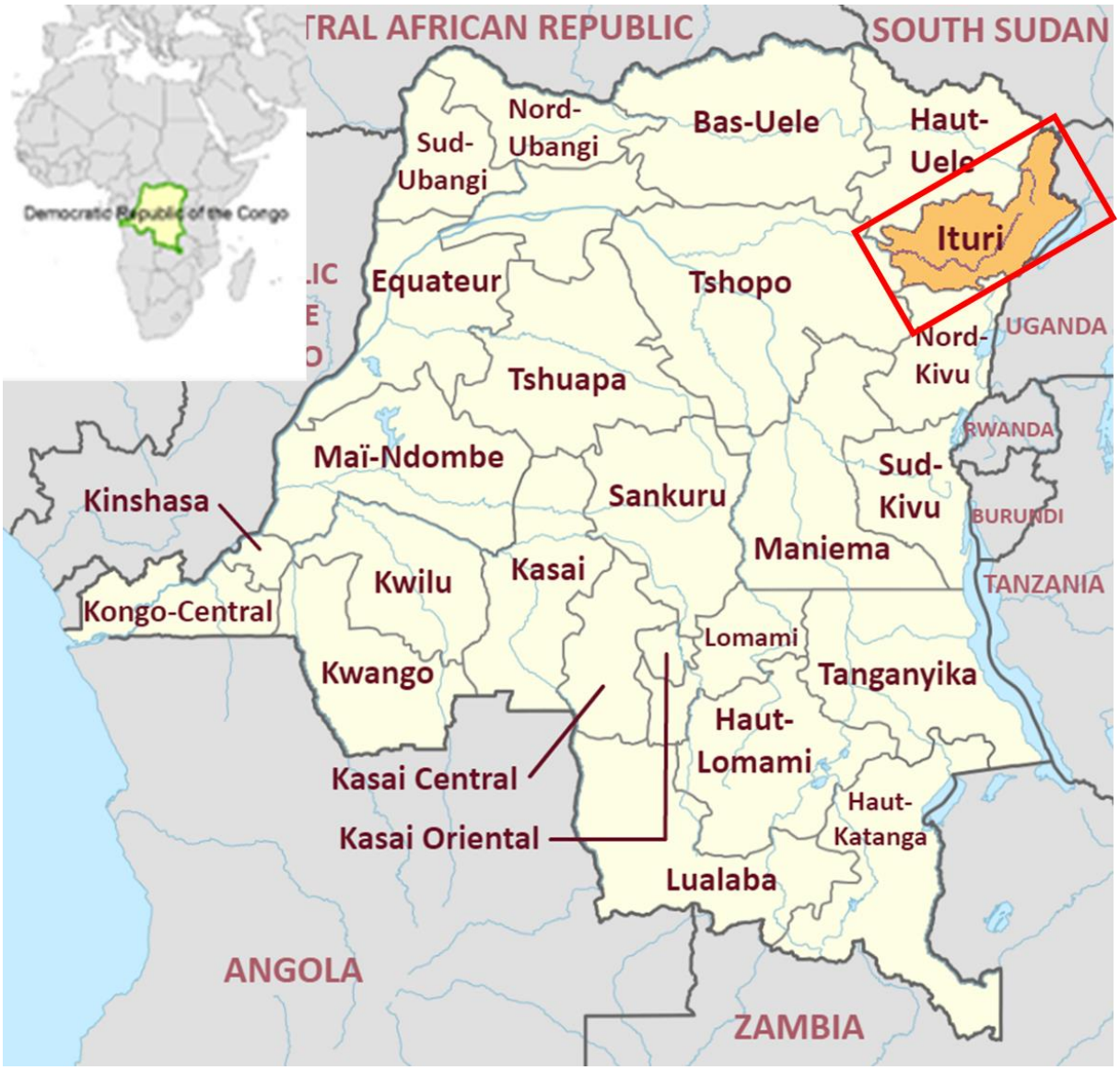

Figure 5.1. Study site: Ituri province among the 26 provinces of DRC. Map of Democratic Republic of the Congo in the centre of Africa, and Ituri province, situated among the 26 provinces, in the northeastern part of the country.

Study design and population

Two community–based studies were carried out across Ituri province during the long school holidays corresponding to the second dry season of the year (June to July) so as to include as much as possible children and their families in the study. First, in 2016, a cross–sectional study was carried out in 46 randomly selected villages to assess the geographical distribution of *S. mansoni* infection prevalence and infection intensity. Second, in 2017, an in–depth, cross–sectional study was conducted in 12 purposively selected villages to identify the risk factors. For both studies, people aged one year and older were eligible to participate.

The 2016 cross–sectional study used a two–stage random sampling procedure. First, 12 health districts were randomly selected across the province. Second, two to five villages were randomly selected per health district, proportional to the density of the population. Fifty to 100 participants were enrolled in each village.

For the 2017 in–depth study, twelve health districts with high *S. mansoni* prevalence were purposively selected. Three of the selected health districts could not be visited due to security concerns. In each of the remaining nine health districts, two to five villages were randomly selected. In each village, 10 to 30 households were randomly selected. In total, 144 households were visited. In each household, all members present on the survey date (aged one year and older) were invited to participate.

The chosen villages (communities) in 2016 and 2017 belong to specific health areas and in each health area there is a health centre that meets the criteria of geographical accessibility.

In both studies, unique reference codes were assigned to each study participant. Assuming a mean schistosomiasis prevalence of 50.0% in this population, we calculated that 100 to 150 individuals should be included per health district, for each study.

Assessment of *S. mansoni* infection

In 2016, study participants provided one stool sample from which *S. mansoni* could be diagnosed using a duplicate Kato–Katz test (2 smears per stool) [79]. One hour after collection, stool specimens were transported to the local laboratory for examination. The smears were allowed to clear for half an hour before being read. The number of eggs detected on the smear was recorded for each helminth species separately.

Urine samples were requested from 20.0% randomly selected participants of each village. The centrifugation pellets of these urine samples were microscopically examined (40X magnifier objectives). Before discarding the urine samples, strip tests were also performed (Combina™ 10 M, Human Diagnostics Uganda).

In 2017, participants provided stool samples on five consecutive survey days to assess the compliance of the participants to provide multiple samples, but also to evaluate the improvement of the sensitivity of the Kato-Katz technique according to the number of stool samples examined. On the last day, participants were asked to provide a urine sample as well. Both stool and urine samples were transported to the local laboratory for processing within an hour of collection. A duplicate Kato–Katz test [79] was performed (2 smears per stool) on each stool sample. Again, the number of eggs detected on the smear was recorded for each helminth species separately. In addition, an *S. mansoni* –based point–of–care cathodic circulating antigen (POC–CCA) test [144] was carried out on the urine samples. We combined the Kato-Katz and POC-CCA technique to overcome the weakness and shortcomings of the former technique, through the strength and quality of the latter, and *vice versa*.

To ensure quality control, about 10-30% randomly selected Kato-Katz slides were re-read by the principal investigator. In practice, every tenth negative and positive slides were put aside for re-examination. All the positive and negative slides were confirmed. However, there were some small differences in egg count when the number of eggs was high. The microscopist repeated his count. When he found the same result as before, the mean value of the two count of the microscopist and that of the principal investigator was taken in account. In two cases, one of very high intensities of *Ascaris* and another of *S. mansoni*, all the three microscopists and the principal investigator counted the number of eggs separately. Then, the mean value of all was considered. In another one

case, the principal investigator found a hookworm egg that was missed by the microscopists. Urine POC-CCA test trace and weakly positive results were discussed by the laboratory team and the principal investigator.

Questionnaire data

In 2016, demographic information was collected with a short questionnaire addressed to each study participant, while both a household and an individual questionnaire was used in 2017. The head of household answered the household questionnaire. It included questions regarding the number of household members, the availability and quality of sanitation (latrine), the source and type of water used, the building material of the house, household goods, estimated monthly income and the existence of mass drug administration (MDA) for the benefit of the villagers. The individual questionnaire addressed demographic details (age, sex, tribal group, religion, main occupation, education, and usual defecation place), knowledge of schistosomiasis (disease, transmission, prevention, and treatment) and potential risk factors (exposure to water, socioeconomic status, time of residence, lack of sanitation and safe drinking water, etc.).

Data management

In both studies, data were entered in Excel and cross checked with the source data. Data management and data analysis were performed with Stata, version 14.2 (Stata Corp LP; College Station, USA). Age groups were defined as follows: (i) 1–4 years, (ii) 5–9 years, (iii) 10–14 years, (iv) 15–19 years, (v) 20–29 years, (vi) 30–39 years, (vii) 40–49 years, (viii) ≥50 years to highlight as much as possible the level of infection in infants, children, younger and older adolescents, as well as young adults, adults, and older people. Body mass index (BMI) was calculated and four BMI categories were established: underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30 kg/m²). Using the arithmetic mean of the egg positive stool samples, *S. mansoni* infection intensity (eggs per gram [EPG]) was categorized as light (1–99 EPG), moderate (100–399 EPG), and heavy (≥400 EPG) [79, 143].

Figure 5.2: Study field procedures. Enrolment of study participants, administration of household and individual questionnaires, anthropometric measurements, and collection of stool samples.

Statistical analysis

Descriptive statistics (means and frequencies) were used to summarize continuous and categorical variables, respectively. The chi–square test (χ^2) and Fisher exact test were used to compare proportions. An univariable logistic regression analysis was performed to associate *S. mansoni* infection (outcome) with potential risk factors (predictors), such as demographic, geographical, behavioural, and socioeconomic variables. Co–variables exhibiting an association at a significance level of at least 20%, as determined by the likelihood ratio test (LRT), were included in the multivariable logistic regression models. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. To visualize prevalence rates at health district and village levels, a twoway scatter bar command, which displays numeric (y, x) data as histogram–like bars, was performed using the vertical bar plot option in STATA. Bars were drawn at the specified xvar values (health districts and villages) and extended up from 0 according to the corresponding yvar values (prevalence). To explore the relationship between *S. mansoni* infection risk and age, age– and sex–prevalence curves were produced using the twoway quadratic fitted values. This command calculates the prediction for yvar (prevalence or intensity) from a linear regression of yvar on xvar and xvar^2 and plots the resulting curve. An *S. mansoni* distribution map was made as follows: village prevalence (resulting from the Kato–Katz tests in 2016 and 2017) and geographic decimal coordinates (latitude and

longitude) were entered in two adjoining columns in Excel and then plotted. A non–uniform spline interpolation was performed with MATLAB®, revealing shadows, the intensity of which were proportional to prevalence levels. Dark coloured shadows indicate high prevalence and lighter shadows (high transparency) indicate low prevalence. *P*–values below 5% were considered statistically significant.

5.5 Results

Study population

Of the 2,322 individuals enrolled in 2016, 2,131 participants completed all examinations and were included in the final analysis (Figure 3). Participants represented 46 villages, with at least 10 participants from each village except for Ramogi village (Angumu health district), where only 6 participants were included (Table S5.1d). Table 5.1 shows the demographic characteristics of participants in the *S. mansoni* geographical distribution study. There were slightly more female (51.0%) than male participants. The mean age of participants was 22.2 years, with little difference between men and women. Children under 15 years made up almost half of all study participants. Nearly two thirds of participants (58.0%) had a normal BMI, one third (33.0%) were underweight and nine percent were overweight or obese. More than three quarters of the overweight/obese group were female.

Of the 1,044 individuals enrolled in the 2017 study, 707 completed all procedures (Figure 3). Participants lived in one of 12 villages (144 households), with at least 23 participants present in each village (Table S5.2a). Table 5.2 shows the demographic characteristics of participants in the in–depth study carried out in 2017.

Female participants (56.3%) outnumbered male participants. More than half of the participants (51.4%) were younger than 15 years. Almost half of the participants (49.4%) were underweight, 38.3% had a normal weight, and 12.3% were overweight or obese. More than 80% of the overweight/obese group were female.

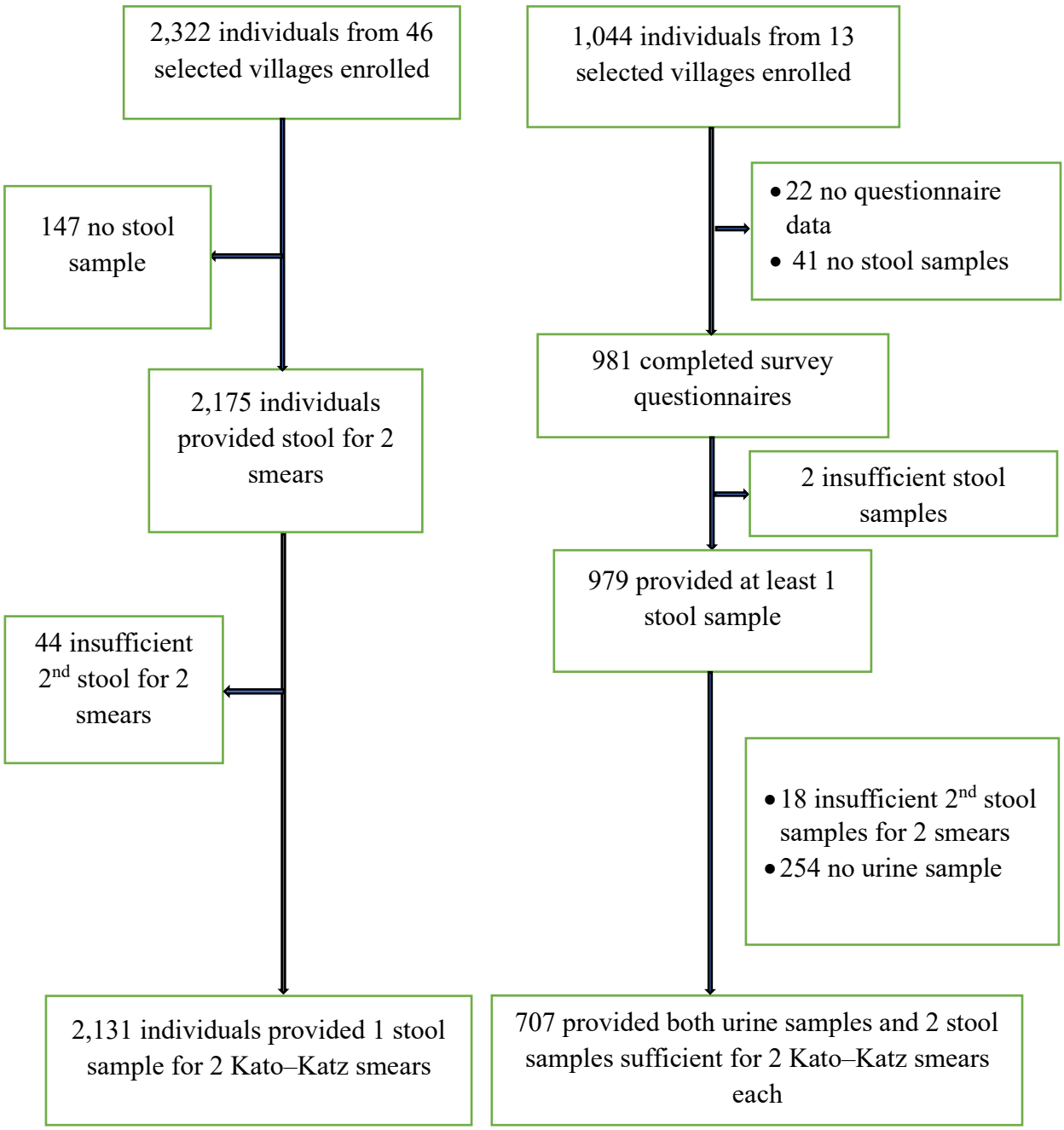

Figure 5.3. Study participants' inclusion flowchart for the Ituri schistosomiasis surveys. Left: In 2016, geographical distribution study in 46 villages; Right: In 2017, in–depth study in 12 villages.

Table 5.1. Geographical distribution study 2016: Demographic characteristics of the participants. Cross–sectional study of geographical distribution of *S. mansoni* infections, conducted in 46 randomly–selected villages in 12 of the 36 health districts in Ituri province (n=2,131).

Characteristics	Subgroups	Total n (%)	Female n (%)	Male n (%)
Overall n (%)		2,131 (100)	1,087 (51.0)	1,044 (49.0)
Mean				
Age (years)		22.2	22.8	21.6
Body Mass Index (BMI)				
Obese	(BMI≥30.0)	35 (1.6)	30 (85.7)	5 (14.3)
Overweight	(BMI=25.0–29.9)	157 (7.4)	121 (77.1)	36 (22.9)
Normal weight	(BMI=18.5–24.9)	1,235 (58.0)	634 (51.3)	601 (48.7)
Underweight	(BMI<18.5)	704 (33.0)	302 (42.9)	402 (57.1)
Children	(<18 years)	1,107 (52.0)	507 (45.8)	600 (54.2)
Adults	(≥18 years)	1,024 (48.0)	580 (56.6)	444 (43.4)
Age categories (years)				
1 – 4		26 (1.2)	12 (46.2)	14 (53.8)
5 – 9		436 (20.5)	202 (46.3)	234 (53.7)
10 – 14		496 (23.3)	228 (46.0)	268 (54.0)
15 – 19		241 (11.3)	118 (49.0)	123 (51.0)
20 – 29		337 (15.8)	208 (61.7)	129 (38.3)
30 – 39		236 (11.7)	132 (55.9)	104 (44.1)
40 – 49		199 (9.3)	115 (57.8)	84 (42.2)
≥50		160 (7.5)	72 (45.0)	88 (55.0)
Residence				
Rural		1 951 (91.6)	992 (50.8)	959 (49.2)
Urban		180 (8.4)	95 (52.8)	85 (47.2)
Ethnic groups				
Nilo–Hamites		106 (5.0)	48 (45.3)	58 (54.7)
Bantu		398 (18.7)	222 (55.8)	176 (44.2)
Nilotic		753 (35.3)	368 (48.9)	385 (51.1)
Sudanese		582 (27.3)	293 (50.3)	289 (49.7)
Pygmies		292 (13.7)	156 (53.4)	136 (46.6)

Table 5.2. In–depth study 2017: Demographic characteristics of the participants. Study conducted in 12 purposively–selected villages in 6 of the 36 health districts in Ituri province (n=707).

Characteristics	Subgroups	Total n (%)	Female n (%)	Male n (%)
Overall n (%)		707 (100)	398 (56.3)	309 (43.7)
Mean				
Age (years)		21.0	21.6	20.2
Family size (no)		8.6	8.5	8.6
Body Mass Index (BMI)				
Obese	(BMI≥30.0)	63 (8.9)	51 (81.0)	12 (19.0)
Overweight	(BMI=25.0–29.9)	24 (3.4)	23 (95.8)	1 (4.2)
Normal weight	(BMI=18.5–24.9)	271 (38.3)	158 (58.3)	113 (41.7)
Underweight	(BMI<18.5)	349 (49.4)	166 (47.6)	183 (52.4)
Children	(<18 years)	406 (57.4)	205 (50.5)	201 (49.5)
Adults	(≥18 years)	301 (42.6)	193 (64.1)	108 (35.9)
Age categories (years)				
1 – 4		61 (8.6)	29 (47.5)	32 (52.5)
5 – 9		158 (22.4)	77 (48.7)	81 (51.3)
10 – 14		144 (20.4)	73 (50.7)	71 (49.3)
15 – 19		68 (9.6)	40 (58.8)	28 (41.2)
20 – 29		83 (11.7)	67 (80.7)	16 (19.3)
30 – 39		75 (10.6)	49 (65.3)	26 (34.7)
40 – 49		56 (7.9)	32 (57.1)	24 (42.9)
≥50		62 (8.8)	31 (50.0)	31 (50.0)
Residence				
Rural		377 (53.3)	216 (57.3)	161 (42.7)
Urban		330 (46.7)	182 (55.2)	148 (44.8)
Ethnic groups				
Nilo–Hamites		160 (22.6)	98 (61.3)	62 (38.7)
Bantu		312 (44.1)	179 (57.4)	133 (42.6)
Nilotic		166 (23.5)	88 (53.0)	78 (47.0)
Sudanese		69 (9.8)	33 (47.8)	36 (52.2)

Schistosoma mansoni infection prevalence and intensity

Table S1a summarizes results of the two surveys for the different diagnostic approaches. The global results are shown as prevalence and intensity of infection with the 95% confidence intervals (95% CI). Detailed analysis of the results by health districts, villages, sex, age groups, residence, ethnic groups, and altitude are then shown through Tables S5.1b-S5.1d and S5.2a-S5.2i.

Prevalence

In 2016, the overall *S. mansoni* average prevalence measured by Kato-Katz was 40.0% (37.9-42.1) (Tables S5.1a) ranging from 3.9% to 80.2% across the 12 health districts. Detailed results showed significant variation of infection prevalence between sex, age categories, ethnic groups, altitude levels, villages, and health districts. Infected individuals were found in 43 of the 46 (93.5%) of the investigated villages. Male study participants (42.6%) were more frequently infected than females (37.4%) (p=0.015). The difference in the prevalence of infection across the eight age groups (Table S5.1b) and in both sexes (Table S5.1c) was highly significant (p<0.001). The prevalence in the youngest age group (1-4 years) was a quite low (7.7%), being 8.3% and 7.1% in girls and boys, respectively. This figure rose clearly in subsequent age groups to reach a maximum of 50.2% in the 15-19 years, being 45.8% in females and 54.5% in males. Then, the prevalence declined slightly after this peak but remained generally high throughout.

Among the twelve health districts surveyed, *S. mansoni* infection was highest in the health districts of Nyarambe (80.2%), Tchomia (73.7%), and Angumu (70.2%) all situated at the shore of Lake Albert. They are followed by Lolwa (52.5%), Komanda (50.0%), Nyankunde (49.2%), and Bunia (33.9%) situated in the central and south-western regions of the province. For the rest of the health districts situated in the high-hill region (Bambu, Rethy, Logo) or in the northern region (Adi and Laybo), the overall prevalence was less than 10.0%. The difference in the prevalence of infection across the twelve health districts was highly significant (p<0.001) (Table S5.1d and Figure 5.4). The difference in the prevalence of infection according to the altitude above the sea level – high (>1,800 m), middle (1,000-1,800 m), and low (<1,000 m) – was highly significant in both sexes (p<0.001) (Table S5.1b and Figure 5.8). However, the difference between rural areas (40.5%) and urban area (33.9%) (p=0.081), and both among females (p=0.568) and males (p=0.060), was not significant. At the village level, infection prevalence varied widely but was

mostly consistent with the health district mean value. In three of the 46 villages, no *S. mansoni* infection was detected. These villages were Upepeni and Ombanya, in the Adi and Laybo health districts, respectively (northern Ituri province), and Fundi, in the Rethy health district (located in the hills at about 1,920 m above sea level). Villages along the shore of Lake Albert had prevalence rates over 70%, with the highest prevalence rate reaching 90.2% in Kolokoto (at the Ugandan border), in the Nyarambe health district (Table S5.1d and Figure 5.5).

As for the ethnic groups, the prevalence of S, mansoni infection was highest in Pygmies (50.3%), followed by Bantu (48.0%), Nilotic (47.4%), and Nilo-Hamite (47.2%). The Sudanese ethnic group had the lowest (18.4%) (Table S5.1b), and all these differences were consistent with sex (Table S5.1c) and were statistically significant (p<0.001).

In 2017, the overall *S. mansoni* prevalence was 38.5% with one stool sample 55.0% with two stool samples examined by Kato–Katz test alone. The prevalence rose to 62.5% with one POC-CCA test alone and to 73.1% after combining the Kato–Katz results of two stool samples with one POC–CCA test findings. All the results were consistent in both sexes (Tables S5.2b-S5.2e, and S5.2g- S5.2i). In this section, we only took in account results from the combining diagnostic approach (2KK+POC-CCA) which appeared more relevant in our viewpoint (Table S5.2g). Herein also, detailed results showed significant variation of infection prevalence between health districts, villages (Table S5.2a), and age categories (Table S5.2g) (p<0.001). Infected individuals were found in all the 12 (100%) investigated villages. Males (74.1%) were slightly more infected than females (72.4%) (p=0.603). The difference in the prevalence of infection across the eight age groups was highly significant (p<0.001). This difference was observed both among female (p=0.030) and male (p=0.001) participants (Table S5.2i). The prevalence in the youngest age group (1-4 years) was high (62.3%), being 62.1% and 62.5% in girls and boys, respectively. This figure rose sharply in subsequent age groups to reach a maximum of 86.1% in the 10-14 years, being 84.9% in girls and 87.3% in boys. In males however, the peak reached 93.8% in 20-29 years age category. Then, the prevalence declined after these peaks but remained generally high throughout (Figure 5.4).

S. mansoni infection was high in all the six health districts surveyed, varying between 70.0-95% among the health districts of Tchomia, Bunia, Angumu, Lolwa, and Mandima. The health district of Nia-Nia, located in the extreme southwest of the Ituri province, had the lowest prevalence (52.8%). The difference in the prevalence of infection across the six health districts was highly

significant (p<0.001) (Table S5.2a). The differences in the prevalence of infection according to the altitude above the sea level and rural/urban areas were not significant (p=0.214) both among females (p=0.544) and males (p=0.419). At village level, prevalence varied around the health district mean value (Figure 5.4). The lowest prevalence rate (32.3%) was found in Bankoko, Nia–Nia health district, while the highest rates (over 90%) were all found in the forest region in the southern part of the province (i.e., Mandima, 95.0% and Pekele, 96.2%, in Mandima and Lolwa health districts, respectively) (Table S5.2a).

As regards ethnic groups, Pygmies could not be included in the analysis. The prevalence of *S, mansoni* infection was highest in Sudanese (85.5%), followed by Bantu (73.4%), Nilotic (71.7%), and Nilo-Hamite (68.8%). The differences were not statistically significant (p=0.068) and were consistent with sex (p=0.208) and (p<0.350) among females and males, respectively (Table S5.2g).

Comparing the prevalence of *S. mansoni* infection at the health district level (Figure 5.4), there does not appear to be a great difference between 2016 and 2017 rates for Angumu (70.2% and 73.1%) or for Tchomia (73.7% and 70.0%), both of which are situated on the shore of Lake Albert. However, the difference between 2016 and 2017 rates is discernible for Bunia (33.9% and 70.9%), in the central Plateau region and for Lolwa (52.5% and 93.4%), in the southern forest–covered region of Ituri province.

Intensity of infection

The arithmetic mean intensity of *S. mansoni* infection in the twelve health districts investigated in 2016 and in the six in 2017 are shown in Tables S5.1d and S5.2a, respectively. In 2016, the egg count for *S. mansoni* ranged from 0 to 14,424 epg, and from 0 to 5,472 epg in 2017. The overall arithmetic mean infection intensities were 207.4 epg in 2016, and in 2017, 100.9 epg using 1KK (Table S5.2b) and 104.1 epg using 2KK (Table S5.2d), respectively.

In 2016, six health districts including Nyarambe (928.6 epg), Tchomia (640.1 epg), Angumu (392.3 epg), both located along the shore of Lake Albert, and those of Lolwa (199.3 epg), Komanda (188.9 epg), and Nyankunde (133.4 epg), located in the central and south-western areas, were most heavily infected with *S. mansoni*. The six other health districts had light infections (<100 epg), with 0.7-10.0% of moderate and 0.0-2.1% of heavy *S. mansoni* infections (Table S5.1d). The proportion

of males with heavy infection intensity (13.8%) was higher compared to that of females (9.7%) (p=0.013) (Table S5.1b). The age distribution of *S. mansoni* infection prevalence and intensity is shown in Figure 5.6. In fact, the highest egg counts were found among boys of the age groups of 10-14 years (14,424 epg), 15-19 years (13,248 epg), whereas girls of the age groups of 5-9 years (10,680 epg) and 10-14 years (8,376 epg) bore the heaviest *S. mansoni* infection intensities. The difference in the intensity of infection across the eight age groups was highly significant in both sexes (p<0.001). The figure of infection intensity follows that of the prevalence, rising sharply to peak in 10-19 years age group and declining with the increasing age, but remaining high into the older age. As for the ethnic groups, the heaviest mean intensities in males and females were as follows: among Nilotic: 430.6 and 318.8 epg, Pygmies: 232.3 and 192.6 epg, Bantu: 185.7 and 109.9 epg, and among Nilo-Hamite: 125.0 and 162.0 epg, respectively. The lowest infection intensity was found among Sudanese: 62.6 and 23.7 epg in males and females, respectively (Tables S5.1b and S5.1c).

In 2017, the heaviest infection intensities were found in the health districts of Lolwa (420.5 epg) and Mandima (131.4 epg), in the south-western forest-covered areas, and in Tchomia (109.2 epg) along the shore of Lake Albert. They were followed by Angumu (83.9 epg) and Bunia (48.6 epg). The lowest infection intensity was found in Nia-Nia health district (5.8 epg). Overall, the proportion of males with heavy-intensity infections (5.8%) was lower (not significant) than that of females (6.5%) (p=0.564) (Table S2b). The age distribution of *S. mansoni* infection prevalence and intensity is shown in Figure 6 and Table S2c. The highest egg counts were found among girls 5–9 years (5,472 epg), followed by women aged 30–39 years (2,040 epg), and among boys 5–9 years (4,092 epg) followed by men aged 40–49 years (2,868 epg). The difference in the intensity of infection across the eight age groups was the same as in 2016, highly significant for both sexes (p<0.001), and following the highs and lows of infection prevalence, as described above. However, for the four remaining ethnic groups, the pattern appears reversed. In 2017, Sudanese males (281.0 epg) and females (265.4 epg) had the heaviest infection intensities, followed by Bantu males (103.7 epg) and females (98.3 epg). Among Nilotic and Nilo-Hamite ethnic groups, males had 63.7 and 26.1 epg, and females had 84.1 and 72.0 epg, respectively (Table S2d).

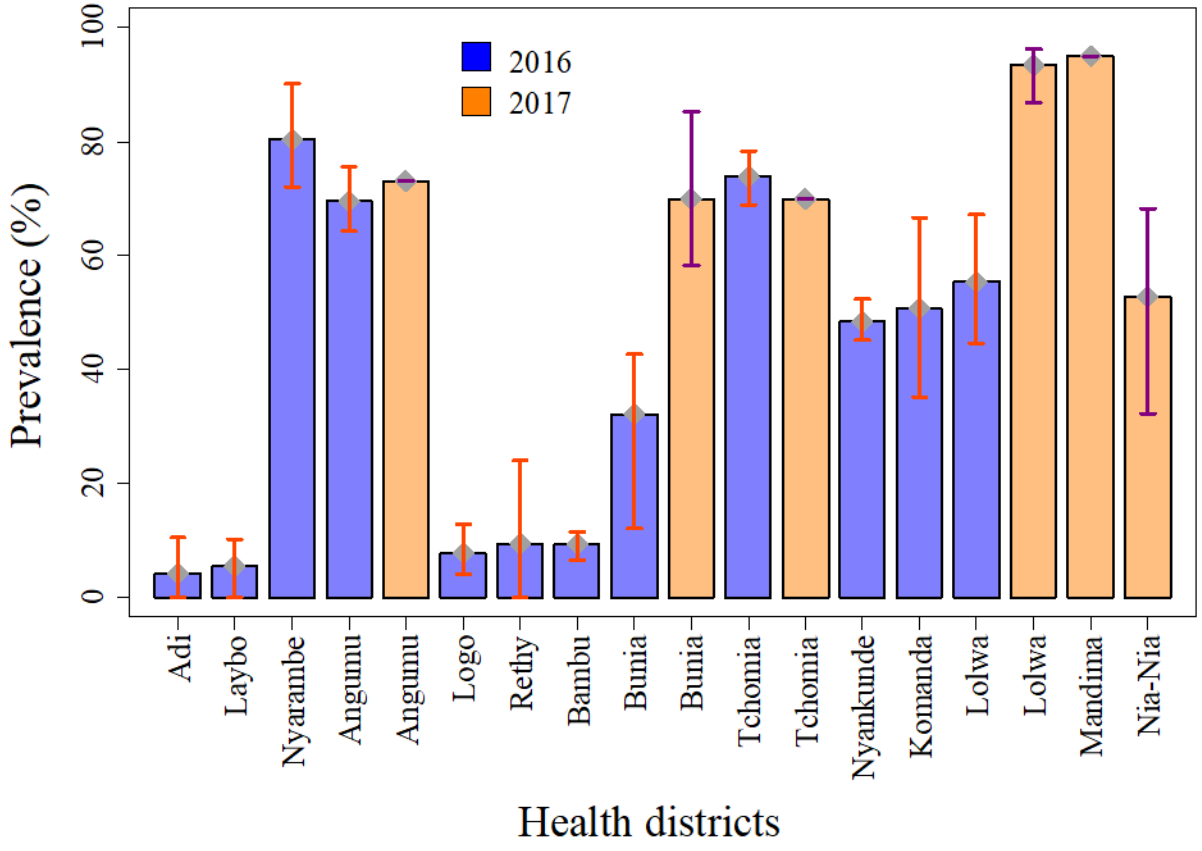

Figure 5.4. *S. mansoni* prevalence by health district in 2016 and 2017. Blue bars: 2016 study (46 study villages across 12 health districts), Kato–Katz test (two smears) of one stool sample. Orange bars: 2017 study (12 study villages across 6 health districts), Kato–Katz test of two stool samples (four smears) and one POC–CCA urine test.

Figure 5.5 shows a map of Ituri province with the *S. mansoni* village prevalence rates observed in the 2016 and 2017 surveys. The rates were generally higher in the South than in the North and were highest in the lowlands and along the shores of Lake Albert.

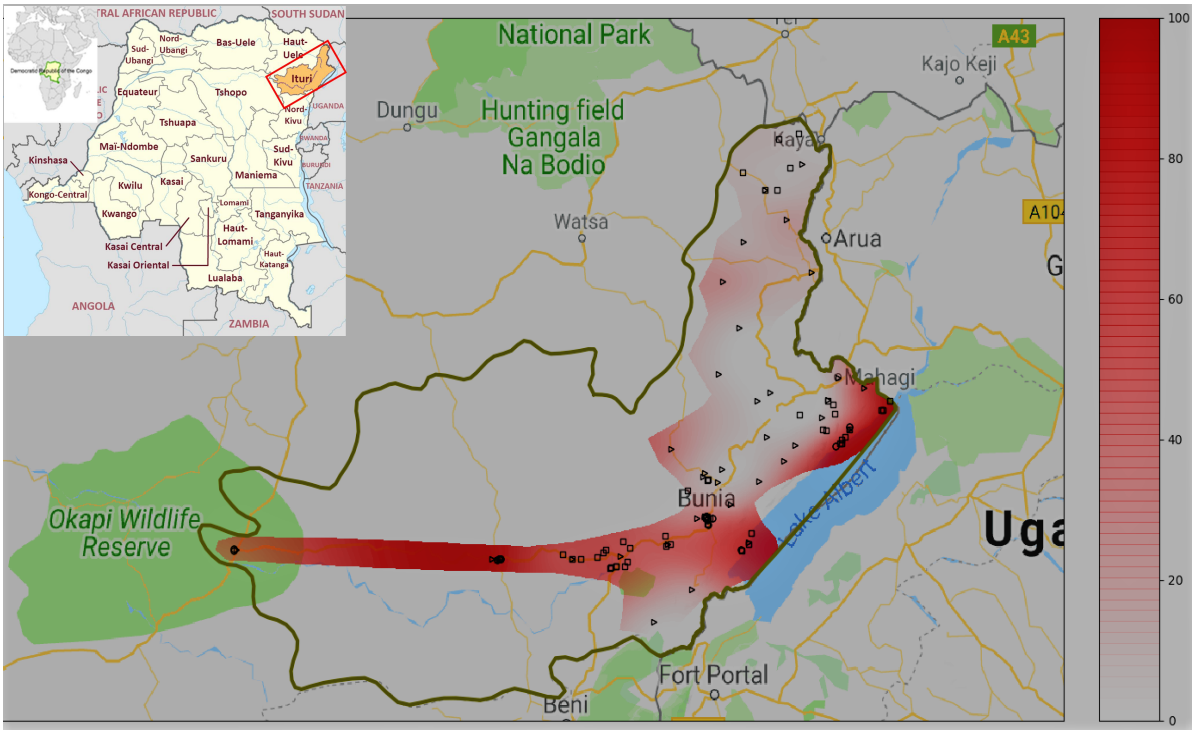

Figure 5.5. Map of Ituri province with estimated *S. mansoni* prevalence. Estimated *S. mansoni* prevalence using non–uniform spline interpolation. Intensity of red shadows is proportional to prevalence levels. Dots indicate studied villages. Areas outside of the red shadow were inaccessible, dense tropical forest and sparsely populated.

The relationship between the risk of *S. mansoni* infection and age is displayed in Figure 5.6. In both the 2016 and 2017 studies, prevalence and intensity increased proportionally with age among both female and male participants until max. 29 years. *S. mansoni* infection prevalence peaked at around 50.0% in 2016, and at around 80.0% in 2017. While prevalence was lower in 2016, peak intensity was higher (207.3 EPG) in 2016, and lower (104.1 EPG) in 2017. Prevalence was highest among children aged 15–19 years in 2016 and decreased among older participants. Prevalence was highest among participants aged 10–14 years in 2017. Overall, the male curves peaked higher in prevalence rates in males than in females in both 2016 and in 2017. Whereas

infection intensity was higher among males than among females in 2016, it was similar among males and females in 2017. The trends for different age groups, in terms of prevalence and intensity, are almost the same. However, a slight difference is observed in 2017: while infection intensity decreased abruptly for males, it remained a plateau for females.

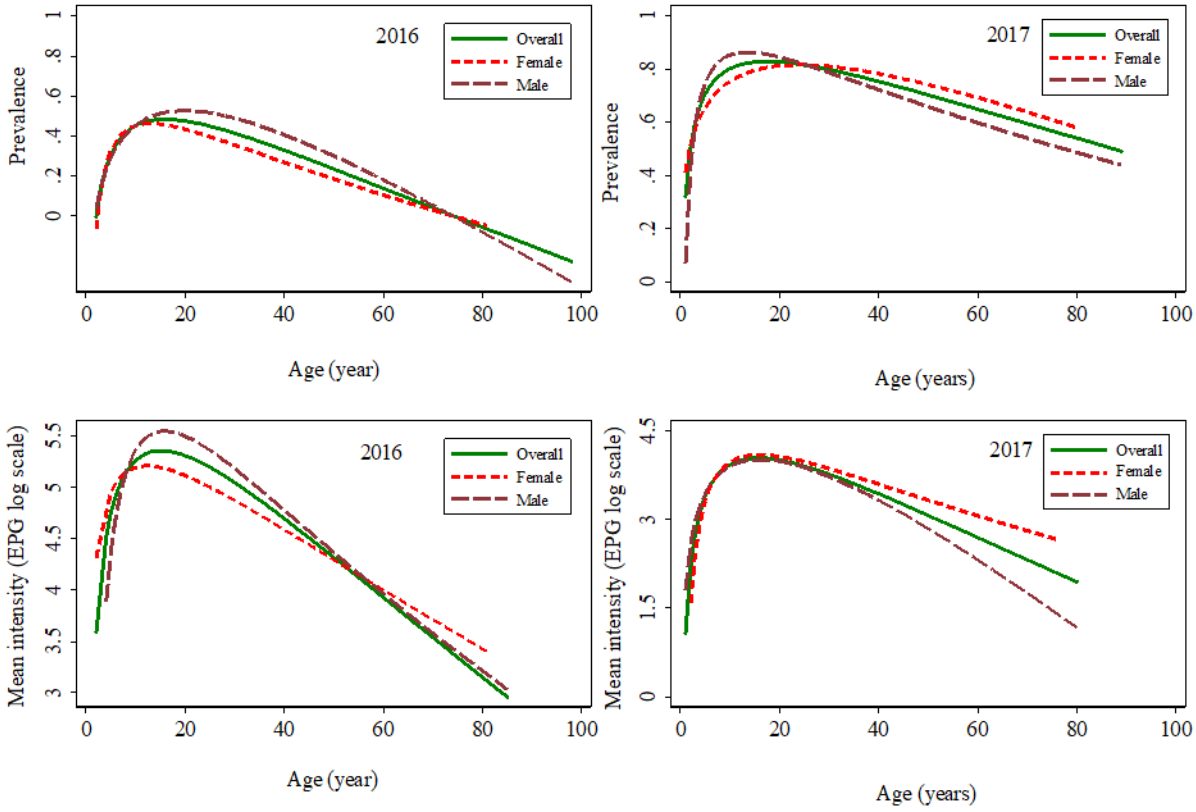

Figure 5.6. Correlation of *S. mansoni* infection prevalence and intensity with age and sex in the two studies. Top: *S. mansoni* infection prevalence by age in 2016 and 2017. Bottom: *S. mansoni* infection intensity (log scale) by age in 2016 and 2017. Lines: Green (solid): all participants; Red (dashed): female; Maroon (long dashed): male.

Correlation between *Schistosoma mansoni* infection prevalence and intensity

The relationship between *S. mansoni* infection prevalence and intensity is displayed in Figure 5.7. *S. mansoni* village–level infection prevalence was positively correlated with infection intensity in the 2016 and 2017 studies.

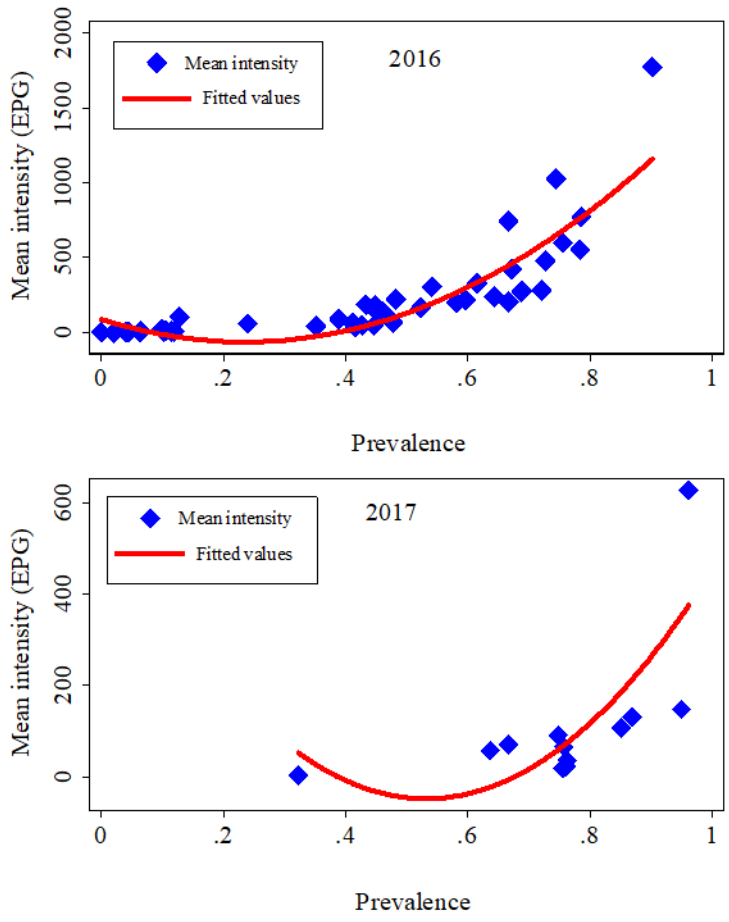

Figure 5.7. Correlation between *Schistosoma mansoni* infection prevalence and intensity at village level in the 2016 geographical study (top) and the 2017 in–depth study (bottom). All quadratic line fitted values.

A relationship was also observed between *S. mansoni* village–level infection prevalence and intensity and altitude in the 2016 study (Figure 5.8). The *S. mansoni* infection prevalence and intensity were negatively correlated with altitude.

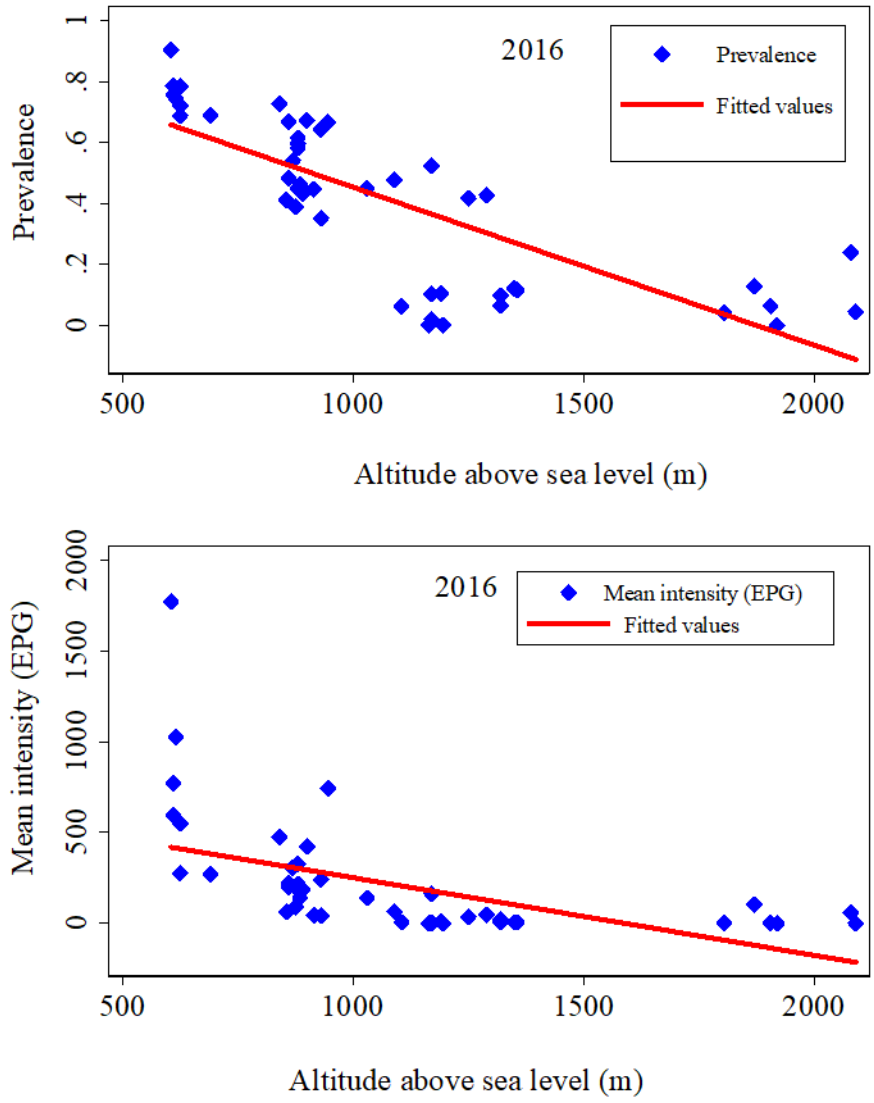

Figure 5.8. Correlation of *S. mansoni* infection prevalence and intensity with altitude (meters above sea level) in the 2016 distribution study. The figure above shows the influence of altitude on *S. mansoni* infection prevalence (top) and intensity expressed as an arithmetic mean (bottom) as observed in 2016. The higher the altitude, the lower the infection prevalence and intensity.

Risk factors for *Schistosoma mansoni* infection

Tables S5.3a–S5.3d show the results of the univariable risk analysis for an *S. mansoni* infection with demographic, socioeconomic, environmental, behavioural, family, and individual variables. A significant increase in risk is associated with age (10–29 years), ethnicity (Sudanese), duration of residence (≥10 years), bad housing, and not owning shoes. *S. mansoni* infection was also associated with living in the health districts of Bunia, Tchomia, Angumu, Lolwa and Mandima, and with proximity of the household to a nearby body of water. The absence of a latrine in the household, washing clothing in streams, and farming were among the most high-risk behaviours. Knowledge about the use of praziquantel was found to be a protective factor against *S. mansoni* infection.

Predictive variables with a significance level of less than 20% in the univariate model were retained in the multivariate risk factor model. Gender was also included in the multivariable logistic regression model (Table S5.4).

Figure 5.9 presents the results of the multivariable risk factor analysis. Ten of 17 variables were significantly associated with *S. mansoni* infection. Participants living in certain health districts, such as Bunia, the unique urban area; Tchomia and Angumu, at the shore of Lake Albert; and Lolwa and Mandima, at the southern and forest–covered region of the province ($p<0.005$) had a significantly increased infection risk. Those with poorly built households ($p<0.044$), or without a latrine ($p=0.022$) had higher odds of being infected. Water contact activities such as swimming ($p=0.014$) and washing clothes in streams ($p=0.018$) were also associated with a significant risk increase. Furthermore, infection risk increased significantly with a longer residence period (≥10 years *vs* shorter period) and closer proximity to water bodies ($p=0.005$). Finally, participants who had a family history of schistosomiasis ($p=0.030$) and those with knowledge of praziquantel treatment had a significantly lower *S. mansoni* infection risk ($p=0.003$).

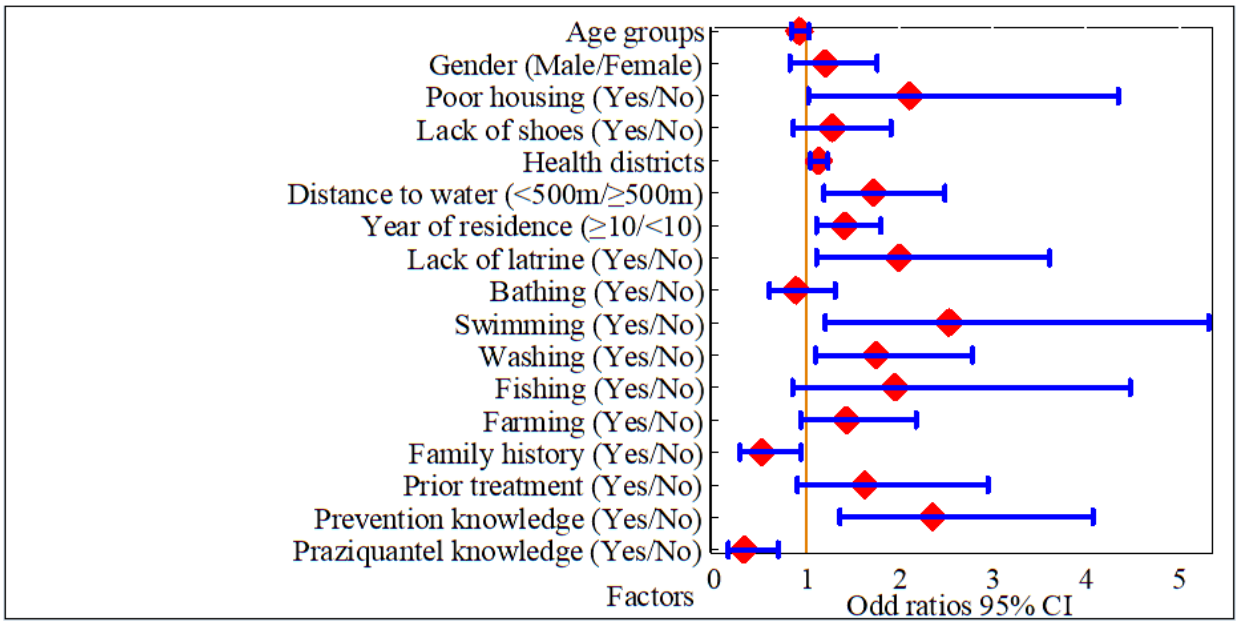

Figure 5.9. **Results of multivariable logistic regression analysis for *S. mansoni* infection in 2017.** Risk analysis performed for participants from 12 villages in Ituri province (n=707). Odds ratios (OR), large red diamond and 95% confidence interval (CI), range indicated by horizontal blue lines.

5.6 Discussion

To the best of our knowledge, this study is the first comprehensive, province–wide assessment of *S. mansoni* infection prevalence, intensity, and risk factors in Ituri since colonial times. In two studies, encompassing 14 (38.9%) of the 36 health districts in Ituri province (Figure S5.2b), including 56 villages and more than 2,800 participants, we firstly used Kato-Katz test alone in 2016 and, alarmed by the findings, applied a more sensitive diagnostic approach which combined both Kato-Katz and POC-CCA tests results and found a very high *S. mansoni* infection burden, revealing a major public health problem in the province.

In the 2016 study, conducted in 46 villages across 12 health districts, 40.0% of the study participants tested positive for *S. mansoni*. First, men showed a higher infection prevalence and displayed heavier infection intensity than women and *S. mansoni* infection was evidently acquired early in the life as many children below 5 years were found infected. Both prevalence and intensity peaked in the 10 – 14 and 15 – 19 years age groups. These results may reflect, on one hand, the more frequent exposure of men to contact with water than women through fishing and farming activities, because culturally in Ituri, men are responsible for meeting the food, clothing and financial needs of their families, while women, on the other hand, are more concerned with domestic needs such as fetching water, washing clothes and dishes, caring for children and preparing food. Thus, they have less contact with water than men. In endemic areas, individuals, mainly children, spend long hours swimming, playing, bathing, or fetching water from water bodies that may contain cercariae. They also defaecate indiscriminately in the environment, causing most of the contamination and increasing their own risk to infection [311].

Second, considerable variability in infection prevalence was observed at the health district level, ranging from 3.9% in Adi to 80.2% in Nyarambe. Three villages were found free of S. mansoni infection, two in the northern side and one in the high hill region. For the two first villages, we did not find an evident reason since these villages are all built near streams that are currently used for washing and bathing. However, our results are consistent with the low *S. mansoni* infection rate in colonial times, when Aru territory prevalence was reportedly below 3.0% [123, 312]. Concerning the village of Fundi, the high altitude with generally cold temperature and water velocity would not be suitable for snail's reproduction. Due to the same reason, people may also

have fewer contacts with water, thereby reducing the probability to become infected [313, 314]. In general, we observed higher infection rates in the south and east of the province compared to the north and west. Data from colonial times [118, 315] and from the mid–1980s [131] showed a similar geographical pattern. Indeed, exposure to the waters of Lake Albert explain this distribution pattern to a large extent. *Biomphalaria* molluscs are abundant in the lake's shallow waters and inhabitants of the lake–side villages intensively use the water for various activities. We also found a negative association between *S. mansoni* infection and altitude. In the colder hillside areas, *Biomphalaria* mollusc development decelerates and human water contact is less frequent, leading to reduced transmission of *S. mansoni* infection [316]. Hence, the Blue Mountains chain of Ituri contributes to a lower infection prevalence pattern in the east of the province.

Surprisingly, *S. mansoni* infection prevalence was very high in the villages situated in the southwestern forest–covered part of the province, i.e. the prevalence in Lolwa and Mandima health districts (Figure S5.2b) exceeded the rates recorded at the lakeshore. However, this region is situated in the lowlands and the population depends on the existing water bodies in the area.

We observed that prevalence was slightly higher in rural areas (75.1%) compared to urban areas (70.9%). Schistosomiasis particularly affects poor communities [280, 317-319] without the means to protect themselves from risky water contacts. Hence, rural communities are especially affected [320-324], but suburban and urban poor communities should also be considered [325-327]. Thus, it is not surprising that the participants living in Ngezi village in Bunia city had a very high *S. mansoni* infection burden, comparable to villages on the shore of Lake Albert. In fact, Ngezi village is situated along the Nyamukau and Ngezi rivers, where adequate water supply and sanitation is lacking.

Infection prevalence was lowest in the health district of Nia–Nia, in the southwestern corner of the province. The remoteness of the villages and the scarcity of water explain the low infection rate. Furthermore, the intermediate host snail *Biomphalaria alexandrina stanleyi* described in this region [328] is thought to be less effective in transmitting *S. mansoni*.

We would expect the infection intensity to show a similar geographical distribution pattern as the infection prevalence. Indeed, in both the 2016 and 2017 studies, we observed a positive association between infection prevalence and intensity at the village level. Similar observations

have been made in other endemic settings for *S. mansoni* [320] and other *Schistosoma* species [329]. In general, variable transmission intensity is thought to account for these observations [330].

In 2017, we observed an overall infection prevalence of *S. mansoni* infection of 73.1%; almost double of 2016 (40.0%). Certainly, the different sampling procedure might account for the higher prevalence rate, as we purposely focused on known *S. mansoni* endemic villages. However, in 2017 we also employed a more sensitive diagnostic approach, consisting of an examination of two stools (4 Kato–Katz smears) and a urine sample (POC–CCA rapid test) per study participant. The infection prevalence ranged from 52.7% to 95.0% at health district level and from 32.3% to 96.2% at village level.

The Kato–Katz technique remains the standard method for diagnosing *S. mansoni*, however it has some shortcomings. It has a low sensitivity; it is time consuming and it requires skilled and trained technicians to identify the *S. mansoni* eggs microscopically. In addition, its sensitivity decreases with decreasing infection intensity [78]. The recently developed POC–CCA test [144] offers an alternative method. Its use in our study increased the number of *S. mansoni* patients identified. Of the 707 participants screened for *S. mansoni* using both techniques, 389 (55.0%) tested positive using the Kato–Katz technique and 442 (62.5%) tested positive using POC–CCA. Upon combining the results from the two techniques, 517 (73.1%) participants were diagnosed with *S. mansoni* infections. These observations are corroborated by the results reported by Okoyo and colleagues [331] when comparing the performance of CCA and KK techniques for evaluating *S. mansoni* infection in areas with low prevalence in Kenya. They found that using the CCA technique increased diagnostic accuracy. There is some discrepancy when comparing our results with those of Standley and colleagues [332], who found that CCA and KK techniques had a similar degree of accuracy. Recent publications discuss the specificity of POC–CCA. POC–CCA showed some cross–reactivity with intestinal nematodes and other health conditions, and therefore might overestimate the prevalence [107, 159].

The 2017 in–depth study was primarily conducted to assess the most important risk factors for an *S. mansoni* infection. We examined the demographic, socioeconomic, environmental, behavioural and knowledge risk factors. Among the most important risk factors, our multivariable logistic regression analysis identified socioeconomic factors, such as poor housing; environmental factors, such as living in a risky health districts, in close proximity to water bodies for long time

periods; behavioural factors, such as the lack of a latrine, and swimming and washing in waterbodies; and knowledge factors [311].

In our study, gender was not a risk factor for infection as females and males had similar levels of infection prevalence in both studies, with a difference <2.0% in 2017 and >5.0% in 2016. These results are consistent with those of other authors [30, 52]. In Ituri province, men and women have different types of water contact activities, but contact intensities are comparable.

Age was an important risk factor. *S. mansoni* infection prevalence and intensity follow a typical age peak curve. In both studies, the prevalence and intensity increased with age until it peaked among the groups aged 10–19 years. Interestingly, the infection prevalence peak was higher in 2017 (86.1% *vs* 50.2% in 2016), but infection intensity was lower that year (45 epg *vs* 245 epg in 2016). Indeed, the use of a higher sensitivity diagnostic approach in 2017 is mainly responsible for these observations. Children of these age groups are most prone to have excessive mobility that may expose them to infected water while swimming, playing, bathing, washing clothes or fetching water [63]. Our results resemble the patterns reported by Kabatereine and colleagues [29] when describing the epidemiology of *S. mansoni* infections on the Ugandan side of Lake Albert, and by Tukahebwa and colleagues [52] when investigating *S. mansoni* infection in a fishing community on the shores of Lake Victoria.

A striking finding of our study is that children under five years are highly infected with *S. mansoni* (62.3% in 2017). This demonstrate the early life exposure of young children through bathing and playing in infested waters. A similar finding was reported by Nalugwa and colleagues [333], who described the high *S. mansoni* infection prevalence among preschool children in communities along Lake Victoria in Uganda.

Some health districts presented a higher risk of *S. mansoni* infection than others. *S. mansoni* is a focal parasite, meaning the more time people spend in affected areas, the more they are likely to get infected [118, 122].

Some positive associations were likely linked to both behavioural and environmental covariates, such as swimming and washing clothes in streams, which are widely practiced in the province and result in exposure to safe water.

Our study has some limitations. First, the sampling techniques during the two studies were not the same. In 2016, by sampling the first arrivals in the centre of the village, a selection bias may have occurred. Likewise, the population, especially in rural areas, is generally reluctant about research. They adhere more easily to interventions than to prior investigations. Thus, the study compliance in some villages was quite low. Men, in particular, adhered less strictly to the procedures. This difficulty resulted in fewer study participants in some villages. Second, we did not use the same diagnostic approach in each of the two study years. Also, the POC-CCA test was partially used in 2017 and we did not include molecular tests such as polymerase chain reaction (PCR) and/or loop-mediated isothermal amplification (LAMP) that were not available at the time in the region. Due to the prevailing insecurity due to armed groups in the province in 2016, we could only spend a maximum of two days in each village. Therefore, only one stool sample could be collected from each study participant. Given the low sensitivity of the diagnostic approach employed in 2016, we underestimated the true infection rates. Third, the risk factor analysis should be interpreted with caution, as the in–depth study did not include health districts and villages from the northern or hilly regions. Hence, some relevant risk factors might be missing. Fourth, we did not simultaneously conduct a malacological survey to assess the prevalence and rates of cercarial infection of snails in the region. This may have deprived us of valuable information on the transmission of the disease and its seasonality.

The DRC's national NTD control program had completed mapping schistosomiasis and STH in 99.2% of the country's health districts in 2015. It had launched a master plan 2016–2020 that work toward eliminating schistosomiasis as a public health problem by 2025, following the WHO guidelines. These guidelines depend on the level of infection as assessed in school age children (SAC). The subsequent recommendations are that preventive-chemotherapy (PCT) mass drug administration (MDA) be administered: i) annually for SAC and high-risk adults (HRA) in highly endemic communities (prevalence >50.0% in SAC); ii) every-other-year for SAC and selected high-risk adults in moderate to high prevalence communities (10-49.0%); iii) for SAC just twice during their primary school years in communities where prevalence is low (<10.0% in SAC) [287]. Another objective of the DRC NTDs program is to interrupt transmission by increasing access to adequate sanitation and drinking water and by improving the immediate environment of communities [134]. In Ituri province, control activities are performed by the provincial NTD branches situated in Aru and Bunia, which implement the control activities in the northern and

southern health districts, respectively. To the best of our knowledge, only three health districts in the shore of Lake albert, namely Tchomia, Angumu, and Nyarambe, are benefiting the first strategy and all the others use the latter strategy of treating or have not started PCT yet. As the country is vast and without roads or trains, the supply of medicines is a major headache. Health districts routinely obtain their supplies from neighbouring Uganda. Even if the seasonality of transmission were not investigated, the best times for treatment could be during the two annual dry periods, December to February and June to July. During these periods, the weather is warm, and water becomes more and more scarce. People, especially children, make greater use of water courses for play, domestic, recreational, and occupational activities which expose them to infection. Our data will be of practical value to help improve control interventions, especially as COVID-19 became currently a huge global problem. COVID-19. Just to emphasize that this virus will surely make the work to be done even more complicated, not to mention all the people that will get ill and die.

The results of this study are important in several respects. They show that (i) *S. mansoni* is highly prevalent in Ituri province. The infection rates observed are beyond those reported in the 1960s [118] and those described in other parts of the country [53, 62, 68, 126, 127]. The observed infection levels are comparable with those in Ugandan fishing villages [30, 52]. The results also show that (ii) the age distribution of infection prevalence and intensity follows the typical pattern of an *S. mansoni* endemic area, where control measures are insufficiently implemented; and that (iii) preschool children bear a high infection burden and, therefore deserve special attention in the control programme.

In conclusion, our results provide comprehensive baseline data showing that *S. mansoni* is highly endemic and is a major health concern in Ituri province, DRC. Infection prevalence and intensity, and its relationship with the prevailing socioeconomic, environmental, and behavioural risk factors reflect intense exposure and alarming transmission rates. Our findings call for more robust plan of action for the control and, in the future, elimination of *S. mansoni* infection in the Ituri province. Intervention strategies could include the full implementation of WHO recommendations for mass drug administration (MDA) by treating communities adequately according to their real need. Furthermore, additional efforts are required to strengthen and expand community-based programs that promote practices aimed at preventing the spread of *S. mansoni* and other parasitic infections. Comprehensive community-based health education, and

implementation of water, hygiene, and sanitation (WASH) programs both in rural and urban areas are of high value, especially in this context of the currently huge global problem of COVID-19 pandemic that will make the work to be done even more complicated, not to mention all the people that will get ill and die. Altogether, these efforts are likely to yield appreciable and sustainable gains in improving health and welfare of the population of Ituri province.

Acknowledgements

We are grateful to all the study participants, both in 2016 and in 2017. Our sincere thanks to the research teams during this two–year period. We thank the Provincial Health Division officers, all the Health District officers, and the Institut Supérieur des Techniques Médicales de Nyankunde staff for their support, as well as the local authorities for their support during fieldwork.

Funding

The study was funded by private funds (Poverty Fund, Switzerland). The funders had no role in study design, data collection or data analysis, decision to publish, or preparation of the manuscript.

Competing interest

The authors have no competing interests.

Supporting information captions

Table S5.1a. *Schistosoma mansoni* infection prevalence and intensity, global results of the two: 2016 and 2017 studies. Results obtained with 1KK in 2016 (n=2,131) and 1KK, 2KK, one POC-CCA, and 2KK+POC-CCA in 2017 (n=707).

Year/Test		Total N	Infected n	Prevalence (95% CI)	EPG (95% CI)
2016					
	1KK	2,131	852	40.0 (37.9–42.1)	207.4 (173.3-241.5)
2017					
	1KK	707	272	38.5 (34.9–42.1)	100.9 (72.0-129.7)
	2KK	707	389	55.0 (51.3–58.7)	104.1 (77.1-131.1)
	One POC-CCA	707	442	62.5 (58.9–66.1)	NA
	2KK+POC-CCA	707	517	73.1 (69.9–76.4)	104.1 (77.1-131.1)

KK= Kato-Katz test (1KK – one stool sample – two smears and 2KK – two stool samples – four smears). POC-CCA= Point-of-care circulating cathodic antigen urine test. 2KK+POC-CCA= approach of combining 2KK with one POC-CCA. NA= not applicable.

Table S5.1b. *Schistosoma mansoni* infection prevalence and intensity in the 2016 geographical distribution study. Study conducted in 46 villages in Ituri province (n=2,131). One stool sample from each study participant was examined with the Kato–Katz test (two smears per stool).

Characteristic	Prevalence n (%)	Infection Intensity n (%)			EPG (Arithmetic)	
	Positive	Light	Moderate	Heavy	Mean	Maximum
Overall	40.0	17.2	11.1	11.7	207.4	14,424
Sex						
Female	37.4	17.2	10.6	9.7	171.6	10,680
Male	42.6	17.2	11.7	13.8	244.6	14,424
p -value	0.015			0.013		
Age group (years)						
1 – 4	7.7	3.9	3.9	0.0	17.5	360
5 – 9	41.3	18.6	11.5	11.2	213.9	10,680
10 – 14	45.0	17.9	11.3	15.7	313.5	14,424
15 – 19	50.2	14.1	15.4	20.8	330.1	13,248
20 – 29	46.6	21.1	13.1	12.5	187.4	3,312
30 – 39	40.3	18.6	15.3	6.4	144.6	7,824
40 – 49	26.1	13.6	5.5	7.0	70.3	1,296
≥50	13.8	11.9	1.3	0.6	11.7	744
p -value	<0.001			<0.001		
Residence						
Rural	40.5	16.6	11.2	12.7	223.4	14,424
Urban	33.9	23.3	10.0	0.6	33.2	624
p -value	0.081			<0.001		
Ethnic group						
Nilo-Hamite	47.2	26.4	10.4	10.4	141.7	3,096
Bantu	48.0	24.6	15.1	8.3	143.5	9,792
Nilotic	47.4	15.0	12.9	19.5	376.0	14,424
Sudan*	18.4	11.0	4.3	3.1	43.0	5,136
Pygmy	50.3	21.6	15.1	13.7	211.1	7,824
p -value	<0.001			<0.001		
Altitude						
>1800 m	8.7	5.2	1.7	1.7	27.8	3,312
1000–1800 m	22.7	14.5	6.0	2.2	41.8	4,080
<1000 m	61.2	22.4	17.4	21.4	377.8	14,424
p -value	<0.001			<0.001		

Table S5.1c: *Schistosoma mansoni* infection prevalence and intensity in the 2016 geographical distribution study. Study conducted in 46 villages in Ituri province (n=2,131). One stool sample from each study participant was examined with the Kato–Katz test (two smears per stool) by sex among different age groups, residence, ethnic group, and altitude.

Sex	**Female**				**Male**			
Characteristics	Prev.	Heavy	Mean	Max	Prev.	Heavy	Mean	Max
Overall	37.4	9.6	171.6	10,680	42.6	13.8	244.6	14,424
Age group (years)								
1 – 4	8.3	0.0	8.0	96	7.1	0.0	25.7	360
5 – 9	43.1	12.9	271.6	10,680	39.7	9.8	164.1	6,672
10 – 14	43.4	11.4	225.5	8,376	46.3	19.4	388.3	14,424
15 – 19	45.8	14.4	195.7	3,336	54.5	26.8	459.1	13,248
20 – 29	42.3	12.0	160.0	3,096	53.5	13.2	231.6	3,312
30 – 39	37.1	5.3	142.5	7,824	44.2	7.7	147.1	2,760
40 – 49	16.5	2.6	33.2	984	39.3	13.1	121.1	1,296
≥50	13.9	1.4	15.7	744	13.6	0.0	8.5	144
p -value	<0.001	<0.001			<0.001	<0.001		
Residence								
Rural	37.7	10.5	184.3	10,680	43.5	15.0	264.0	14,424
Urban	34.7	1.1	39.2	624	32.9	0.0	26.5	288
p -value	0.568	0.017			0.060	0.001		
Ethnic group								
Nilo-Hamite	39.6	12.5	162.0	3,096	53.5	8.6	125.0	1,344
Bantu	46.0	6.3	109.9	4,080	50.6	10.8	185.7	9,792
Nilotic	46.2	16.6	318.8	10,680	48.6	22.3	430.6	14,424
Sudanese	15.4	1.7	23.7	864	21.5	4.5	62.6	5,136
Pygmy	45.5	12.2	192.6	7,824	55.9	15.4	232.3	2,688
p -value	<0.001	<0.001			<0.001	<0.001		
Altitude								
>1800 m	5.6	0.7	9.0	744	11.7	2.8	46.3	3,312
1000-1800 m	22.4	2.5	46.3	4,080	23.0	1.9	37.0	2,088
<1000 m	57.4	17.3	307.4	10,680	65.6	25.6	451.2	14,424
p -value	<0.001	<0.001			<0.001	<0.001		

Table S5.1d: *Schistosoma mansoni* infection prevalence and intensity in the 2016 geographical distribution study. Study conducted in 46 villages in Ituri province (n=2,131). One stool sample from each study participant was examined with the Kato–Katz test (two smears per stool) by village and health district.

Entity	Prevalence		Intensity (%)			EPG (Arithmetic)	
	Total	n (%)	Light	Mod.	Heavy	Mean	Max
Villages							
Overall	2,131	852 (40.0)	17.2	11.1	11.7	207.4	14,424
Ombanya	48	0 (0.0)	0.0	0.0	0.0	0.0	0.0
Upepeni	39	0 (0.0)	0.0	0.0	0.0	0.0	0.0
Fundi	49	0 (0.0)	0.0	0.0	0.0	0.0	0.0
Moze	50	1 (2.0)	2.0	0.0	0.0	0.5	24
Upani-Logo	50	2 (4.0)	4.0	0.0	0.0	1.0	24
Nioka-Foret	45	2 (4.4)	4.4	0.0	0.0	1.1	24
Aubha	47	3 (6.4)	4.3	2.1	0.0	5.6	120
Kpana	47	3 (6.4)	6.4	0.0	0.0	2.0	48
Nyaradha	46	3 (6.5)	4.4	2.2	0.0	5.2	144
Duba	51	5 (9.8)	7.8	0.0	2.0	20.2	864
Laybo	49	5 (10.2)	10.2	0.0	0.0	6.4	96
Iri	38	4 (10.5)	5.3	5.3	0.0	10.1	192
Nyangaray	52	6 (11.5)	11.5	0.0	0.0	5.1	72
Saio	50	6 (12.0)	10.0	2.0	0.0	7.2	120
Umoyo	47	6 (12.8)	2.1	6.4	4.3	103.1	3,312
Ureli	50	12 (24.0)	14.0	4.0	6.0	59.0	912
Mangiva	37	13 (35.1)	18.9	16.2	0.0	40.2	288
Bandilesu	18	7 (38.9)	16.7	16.7	5.6	88.0	672
Lolua	51	21 (41.2)	25.5	9.8	5.9	61.2	816
Ngezi	48	20 (41.7)	31.3	10.4	0.0	35.0	240
Yambi	82	35 (42.7)	26.8	14.6	1.2	48.0	624
Bolombo	90	39 (43.3)	21.1	13.3	8.9	184.7	7,824
Makwange	47	21 (44.7)	31.9	10.6	2.1	46.0	696
Pinzili I	29	13 (44.8)	24.1	10.3	10.3	181.2	2,592
Ndenge	51	23 (45.1)	19.6	11.8	13.7	138.4	2,088
Makayanga	39	18 (46.2)	28.2	10.3	7.7	140.3	1,872
Loyi-Batine	44	21 (47.7)	27.3	15.9	4.6	66.5	768
Mungamba	29	14 (48.3)	17.2	13.8	17.2	220.1	1,584
Singoma	86	45 (52.3)	31.4	14.0	7.0	164.7	4,080
Bandibiso	24	13 (54.2)	16.7	16.7	20.8	306.0	1,752
Shaurimoya	43	25 (58.1)	16.3	30.2	11.6	199.8	2,520
Tindo	57	34 (59.7)	36.8	12.3	10.5	218.5	5,136
Pinzili II	26	16 (61.5)	30.8	7.7	23.1	327.7	2,688
Paluo	42	27 (64.3)	23.8	19.1	21.4	240.0	1,944
Ramogi	6	4 (66.7)	16.7	16.7	33.3	744.0	2,592

Takumanza	24	16 (66.7)	20.8	20.8	25.0	203.0	744
Pekele	64	43 (67.2)	14.1	25.0	28.1	421.9	9,792
Gengere	77	53 (68.8)	39.0	14.3	15.6	267.7	5,400
Kalambo	45	31 (68.9)	20.0	26.7	22.2	277.9	1,776
Pamaya	43	31 (72.1)	30.2	23.3	18.6	278.0	2,328
Jupanyarabi	55	40 (72.7)	18.2	21.8	32.7	476.5	3,576
Kalako	51	38 (74.5)	11.8	19.6	43.1	1,023.5	10,680
Ndawe	45	34 (75.6)	20.0	24.4	31.1	597.9	4,608
Mita	37	29 (78.4)	21.6	18.9	37.8	552.0	3,096
Wikidhi	42	33 (78.6)	9.5	28.6	40.5	772.6	13,248
Kolokoto	41	37 (90.2)	9.8	4.9	75.6	1,770.7	14,424
p-value		<0.001			<0.001		
Health districts							
Overall	2,131	852 (40.0)	17.2	11.1	11.7	207.4	14,424
Adi	127	5 (3.9)	2.4	1.6	0.0	3.2	192
Laybo	144	8 (5.6)	4.9	0.7	0.0	4.0	120
Logo	144	11 (7.6)	4.2	2.1	1.4	34.7	3,312
Bambu	149	14 (9.4)	8.1	0.7	0.7	10.3	864
Rethy	144	14 (9.7)	6.3	1.4	2.1	20.8	912
Bunia	180	61 (33.9)	23.3	10.0	0.6	33.2	624
Nyankunde	181	89 (49.2)	27.1	13.8	8.9	133.4	4,080
Komanda	416	208 (50.0)	23.3	15.1	11.5	188.9	7,824
Lolwa	162	85 (52.5)	22.8	16.1	13.6	199.3	9,792
Angumu	225	158 (70.2)	26.7	19.1	24.4	392.3	5,400
Tchomia	133	98 (73.7)	17.3	21.8	34.6	640.1	10,680
Nyarambe	126	101 (80.2)	16.7	19.1	44.4	928.6	14,424
p-value		<0.001			<0.001		

Table S5.2a: *Schistosoma mansoni* infection prevalence and intensity in the 2017 in-depth study. Study conducted in 12 villages in Ituri province (n=707). When one stool sample from each study participant was taken in consideration for distribution purpose – examined with the Kato–Katz test (two smears per stool) by village and health district.

Entity		Prevalence	Intensity (%)			EPG (Arithmetic)	
	Total	n (%)	Light	Mod.	Heavy	Mean	Max
Villages							
Overall	707	272 (38.5)	21.2	11.6	5.7	100.9	5,472
Bankoko	31	1 (3.2)	3.2	0.0	0.0	1.9	60
Lumumba	36	7 (19.4)	8.3	11.1	0.0	32.7	336
Kindia	84	18 (21.5)	15.5	6.0	0.0	18.3	252
Mangenengene	41	9 (22.0)	22.0	0.0	0.0	8.8	72
Simbilyabo	77	23 (29.9)	18.2	7.8	3.9	70.8	1,560
Sukisa	59	23 (39.0)	30.5	8.5	0.0	28.9	384
Kadjugi	70	28 (40.0)	25.7	8.6	5.7	109.2	4,092
Gupe	119	49 (41.2)	21.0	13.5	6.7	83.9	1,044
Ngezi	74	35 (47.3)	25.7	16.2	5.4	83.5	1,284
Mandima	40	22 (55.0)	15.0	30.0	10.0	131.4	720
Mambau	23	14 (60.8)	39.1	13.0	8.7	150.3	1,680
Pekele	53	43 (81.1)	28.3	24.5	28.3	537.7	5,472
p-value		<0.001			<0.001		
Health districts							
Overall	707	272 (38.5)	21.2	11.6	5.7	100.9	5,472
Nia-Nia	72	10 (13.9)	13.9	0.0	0.0	5.8	72
Tchomia	70	28 (40.0)	25.7	8.6	5.7	109.2	4,092
Angumu	119	49 (41.2)	21.0	13.5	6.7	83.9	1,044
Lolwa	76	57 (75.0)	31.6	21.0	22.4	420.5	5,472
Mandima	40	22 (55.0)	15.0	30.0	10.0	131.4	720
Bunia	330	106 (32.1)	20.3	9.7	2.1	48.6	1,560
p-value		<0.001			<0.001		

Table S5.2b: *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, when one Kato–Katz (KK) smear was examined (total two smears per person). KK overall results taken in account alone.by sex, age categories, residence, ethnic groups, and altitude.

Characteristics	Prevalence	Intensity (%)				EPG (Arithmetic)	
	(%)	Neg	Light	Mod.	Heavy	Mean	Max
Overall	38.5	61.5	21.2	11.6	5.7	100.9	5,472
Sex							
Female	36.2	63.8	18.6	11.8	5.8	102.5	5,472
Male	41.4	58.6	24.6	11.3	5.5	98.7	4,092
p -value	0.155				0.285		
Age group (years)							
1 – 4	19.7	80.3	14.8	4.9	0.0	13.2	216
5 – 9	36.1	63.9	19.0	10.8	6.3	155.7	5,472
10 – 14	50.7	49.3	25.7	16.0	9.3	122.6	1,848
15 – 19	55.9	44.1	25.0	22.1	8.8	118.9	984
20 – 29	42.2	57.8	21.7	14.5	6.0	105.0	1,680
30 – 39	33.3	66.7	20.0	8.0	5.3	80.0	2,040
40 – 49	28.6	71.4	19.6	7.1	1.8	70.7	2,868
≥50	25.8	74.5	21.0	3.2	1.6	24.0	408
p -value	<0.001				0.002		
Residence							
Rural	44.0	56.0	36.9	14.6	9.6	146.6	5,472
Urban	32.1	67.9	34.9	10.9	2.4	48.6	1,560
p -value	0.001				0.001		
Ethnic group							
Nilo-Hamite	35.0	65.0	35.0	8.8	3.1	54.2	1,560
Bantu	37.5	62.5	36.5	13.8	5.8	100.6	5,472
Nilotic	36.7	63.3	37.4	12.1	5.4	74.5	1,308
Sudanese	55.1	44.9	31.9	20.3	17.4	273.6	4,800
p -value	0.027				0.017		
Altitude							
≥1000 m	32.1	67.9	34.9	10.9	2.4	48.6	1,560
<1000 m	44.0	56.0	36.9	14.6	9.6	146.6	5,472
p -value	0.001				0.001		

Table S5.2c: *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, when one Kato–Katz (KK) smear was examined (total two smears per person). KK results taken in account alone.by sex, age categories, residence, ethnic groups, and altitude.

Sex	**Female**				**Male**			
Characteristics	Prev.	Heavy	Mean	Max	Prev.	Heavy	Mean	Max
Overall	36.2	5.8	102.5	5,472	41.4	5.5	98.7	4,092
Age group (years)								
1 – 4	13.8	0.0	8.3	180	25.0	0.0	17.6	216
5 – 9	36.4	5.2	179.1	5,472	35.8	7.4	133.5	4,092
10 – 14	48.0	8.2	105.5	816	53.5	9.9	140.1	1,848
15 – 19	50.0	12.5	132.0	732	64.3	3.6	100.3	984
20 – 29	38.8	6.0	110.9	1,680	56.3	6.3	80.3	528
30 – 39	28.6	6.1	100.2	2,040	42.3	3.9	42.0	480
40 – 49	31.3	0.0	17.3	156	25.0	4.2	142.0	2,868
≥50	22.6	3.2	29.4	408	29.0	0.0	18.6	120
p -value	0.011	0.150			0.004	0.074		
Residence								
Rural	40.3	9.3	149.4	5,472	49.1	8.1	142.8	4,092
Urban	31.3	1.7	47.0	1,560	33.1	2.7	50.7	1,308
p -value	0.064	0.008			0.004	0.018		
Ethnic group								
Nilo-Hamite	35.7	5.1	72.0	1,560	33.9	0.0	26.1	324
Bantu	34.6	4.5	98.3	5,472	41.4	5.3	103.7	4,092
Nilotic	34.1	8.0	84.1	1,044	39.7	3.9	63.7	1,308
Sudanese	51.5	9.1	265.4	4,800	58.3	19.4	281.0	2,868
p -value	0.293	0.702			0.122	0.006		
Altitude								
≥1000 m	31.3	1.7	47.0	1,560	33.1	2.7	50.7	1,308
<1000 m	40.3	9.3	149.4	5,472	49.1	8.1	142.8	4,092
p -value	0.064	0.008			0.004	0.018		

Table S5.2d: *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, two Kato–Katz (KK) smears were examined (total four smears per person). KK overall results taken in account alone.by sex, age categories, residence, ethnic groups, and altitude.

Characteristics	Prevalence	Intensity (%)				EPG (Arithmetic)	
	(%)	Neg	Light	Mod.	Heavy	Mean	Max
Overall	55.0	44.0	35.9	12.9	6.2	104.1	4,498
Sex							
Female	53.0	47.0	33.9	12.6	6.5	100.1	4,162
Male	57.6	42.4	38.5	13.3	5.8	109.3	4,498
p -value	0.224				0.564		
Age group (years)							
1 – 4	27.9	72.1	24.6	3.3	0.0	14.5	197
5 – 9	53.8	46.2	35.4	12.0	6.3	143.6	4,162
10 – 14	66.7	33.3	38.2	16.0	12.5	152.1	4,498
15 – 19	73.5	26.5	39.7	25.0	8.8	124.0	874
20 – 29	65.1	34.9	43.4	13.3	8.4	103.6	1,494
30 – 39	50.7	49.3	36.0	12.0	2.7	69.6	1,978
40 – 49	44.6	55.4	32.1	10.7	1.8	78.8	3,048
≥50	38.7	61.3	32.3	6.5	0.0	23.9	375
p -value	<0.001				<0.001		
Residence							
Rural	61.0	39.0	36.9	14.6	9.6	156.3	4,498
Urban	48.2	51.8	34.9	10.9	2.4	44.6	720
p -value	<0.001				<0.001		
Ethnic group							
Nilo-Hamite	46.9	53.1	35.0	8.8	3.1	43.9	667
Bantu	56.1	43.9	36.5	13.8	5.8	110.3	4,498
Nilotic	54.8	45.2	37.4	12.1	5.4	72.3	874
Sudanese	69.6	30.4	31.9	20.3	17.4	292.2	3,398
p -value	0.016				0.001		
Altitude							
≥1000 m	48.2	51.8	34.9	10.9	2.4	44.6	720
<1000 m	61.0	39.0	36.9	14.6	9.6	156.3	4,498
p -value	0.001				<0.001		

* KK (by Kato–Katz); Only Kato–Katz positive result (at least one S. mansoni egg in at least 1 of 4 smears).

Table S5.2e: *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, two Kato–Katz (KK) smears were examined (total four smears per person). KK results taken in account alone.by sex, age categories, residence, ethnic groups, and altitude.

Sex	**Female**				**Male**			
Characteristics	Prev.	Heavy	Mean	Max	Prev.	Heavy	Mean	Max
Overall	53.0	6.5	100.1	4,162	57.6	5.8	109.3	4,498
Age group (years)								
1 – 4	24.1	0.0	12.8	197	31.3	0.0	16.1	160
5 – 9	50.7	5.2	148.1	4,162	56.8	7.4	139.3	3,451
10 – 14	60.3	13.7	127.4	1,123	73.2	11.3	177.5	4,498
15 – 19	67.5	12.5	144.8	874	82.1	3.6	94.3	732
20 – 29	65.7	7.5	106.2	1,494	62.5	12.5	92.5	643
30 – 39	44.9	4.1	87.9	1,978	61.5	0.0	35.1	288
40 – 49	50.0	0.0	18.3	104	37.5	4.2	159.5	3,048
≥50	38.7	0.0	31.8	375	38.7	0.0	16.0	110
p -value	0.002	0.003			<0.001	0.001		
Residence								
Rural	57.9	10.7	150.4	4,162	65.2	8.1	164.2	4,498
Urban	47.3	1.7	40.5	636	49.3	3.4	49.5	720
p -value	0.034	0.002			0.005	0.012		
Ethnic group								
Nilo-Hamite	46.9	5.1	53.4	667	46.8	0.0	29.0	312
Bantu	54.2	6.2	105.3	4,162	58.7	5.3	117.1	4,498
Nilotic	52.3	6.8	82.9	874	57.7	3.9	60.4	732
Sudanese	66.7	12.1	257.3	3,398	72.2	22.2	324.1	3,048
p -value	0.257	0.563			0.103	0.001		
Altitude								
≥1000 m	47.3	1.7	40.5	636	49.3	3.4	49.5	720
<1000 m	57.9	10.7	150.4	4,162	65.2	8.1	164.2	4,498
p -value	0.034	0.002			0.005	0.012		

Table S5.2f: *Schistosoma mansoni* infection prevalence in the 2017 in-depth study. Study conducted in 12 villages in Ituri province (n=707). From each sample, two Kato–Katz (KK) smears were examined (maximum four smears per person). In addition, each participant provided a urine sample for point–of–care circulating cathodic antigen (POC–CCA) test. Results of the 4 different diagnostic approaches (1KK, 2KK, POC-CCA, and combined 2KK+POC-CCA).

Diagnostic approach		**1KK**	**2KK**	**CCA**	**2KK+CCA**
Entity/ Prevalence	Total	n (%)	n (%)	n (%)	n (%)
Villages					
Overall	707	272 (38.5)	389 (55.0)	442 (62.5)	517 (73.1)
Bankoko	31	1 (3.2)	3 (9.7)	10 (32.3)	10 (32.3)
Lumumba	36	7 (19.4)	12 (33.3)	18 (50.0)	21 (58.3)
Kindia	84	18 (21.5)	32 (38.1)	60 (71.4)	61 (72.6)
Mangenengene	41	9 (22.0)	20 (48.8)	16 (39.2)	28 (68.3)
Simbilyabo	77	23 (29.9)	27 (35.1)	33 (42.9)	43 (55.8)
Sukisa	59	23 (39.0)	40 (67.8)	29 (49.2)	46 (78.0)
Kadjugi	70	28 (40.0)	39 (55.7)	39 (55.7)	49 (70.0)
Gupe	119	49 (41.2)	68 (57.1)	75 (63.0)	87 (73.1)
Ngezi	74	35 (47.3)	48 (64.9)	59 (79.7)	63 (85.1)
Mandima	40	22 (55.0)	31 (77.5)	34 (85.0)	38 (95.0)
Mambau	23	14 (60.8)	19 (82.6)	19 (82.6)	20 (87.0)
Pekele	53	43 (81.1)	50 (94.3)	50 (94.3)	51 (96.2)
p-palue		<0.001	<0.001	<0.001	<0.001
Health districts					
Overall	707	272 (38.5)	389 (55.0)	442 (62.5)	517 (73.1)
Nia-Nia	72	10 (13.9)	23 (31.9)	26 (36.1)	38 (52.8)
Tchomia	70	28 (40.0)	39 (55.7)	39 (55.7)	49 (70.0)
Angumu	119	49 (41.2)	68 (57.1)	75 (63.0)	87 (73.1)
Lolwa	76	57 (75.0)	69 (90.8)	69 (90.8)	71 (93.4)
Mandima	40	22 (55.0)	31 (77.5)	34 (85.0)	38 (95.0)
Bunia	330	106 (32.1)	159 (48.2)	199 (60.3)	234 (70.9)
p-palue		<0.001	<0.001	<0.001	<0.001

Table S5.2g. *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, two Kato–Katz (KK) smears were examined (total four smears per person). In addition, each participant provided a urine sample for point–of–care circulating cathodic antigen (POC–CCA) test. Combined KK+POC-CCA results.

Characteristic	Prevalence	Infection Intensity		
	KK+CCA n (%)	Light n (%)	Moderate n (%)	Heavy n (%)
Overall	517 (73.1)	254 (35.9)	91 (12.9)	44 (6.2)
Sex				
Female	288 (72.4)	135 (33.9)	50 (12.6)	26 (6.5)
Male	229 (74.1)	119 (38.5)	41 (13.3)	18 (5.8)
p-value	0.603			0.564
Age categories (years)				
1 – 4	38 (62.3)	15 (24.6)	2 (3.3)	0 (0.0)
5 – 9	106 (67.1)	56 (35.4)	19 (12.0)	10 (6.3)
10 – 14	124 (86.1)	55 (38.2)	23 (16.0)	18 (12.5)
15 – 19	55 (80.9)	27 (39.7)	17 (25.0)	6 (8.8)
20 – 29	69 (83.1)	36 (43.4)	11 (13.3)	7 (8.4)
30 – 39	50 (66.7)	27 (36.0)	9 (12.0)	2 (2.7)
40 – 49	36 (64.3)	18 (32.1)	6 (10.7)	1 (1.8)
≥50	39 (62.9)	20 (32.3)	4 (6.5)	0 (0.0)
p-value	<0.001			<0.001
Residence				
Rural	283 (75.1)	139 (36.9)	55 (14.6)	36 (9.6)
Urban	234 (70.9)	115 (34.9)	36 (10.9)	8 (2.4)
p-value	0.214			<0.001
Ethnic groups				
Nilo–Hamite	110 (68.8)	56 (35.0)	14 (8.8)	5 (3.1)
Bantu	229 (73.4)	114 (36.5)	43 (13.8)	18 (5.8)
Nilotic	119 (71.7)	62 (37.4)	20 (12.1)	9 (5.4)
Sudanese	59 (85.5)	22 (31.9)	14 (20.3)	12 (17.4)
p-value	0.068			0.001
Altitude				
≥1000 m	234 (70.9)	115 (34.9)	36 (10.9)	8 (2.4)
<1000 m	283 (75.1)	139 (36.9)	55 (14.6)	36 (9.6)
p-value	0.214			<0.001

* KK+CCA, combined any *S. mansoni* positive result (by Kato–Katz and/or by POC–CCA); KK+, only Kato–Katz positive result (at least one S. mansoni egg in at least 1 of 4 smears).

Table S5.2h. *Schistosoma mansoni* infection prevalence and intensity in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, two Kato–Katz (KK) smears were examined (total four smears per person). In addition, each participant provided a urine sample for point–of–care circulating cathodic antigen (POC–CCA) test. Only POC-CCA results.

Characteristic	Prevalence	Infection Intensity		
	POC-CCA n (%)	Light n (%)	Moderate n (%)	Heavy n (%)
Overall	442 (62.5)	254 (35.9)	91 (12.9)	44 (6.2)
Sex				
Female	249 (62.6)	135 (33.9)	50 (12.6)	26 (6.5)
Male	193 (62.5)	119 (38.5)	41 (13.3)	18 (5.8)
p-value	0.978			0.564
Age categories (years)				
1 – 4	33 (54.1)	15 (24.6)	2 (3.3)	0 (0.0)
5 – 9	89 (56.3)	56 (35.4)	19 (12.0)	10 (6.3)
10 – 14	110 (76.4)	55 (38.2)	23 (16.0)	18 (12.5)
15 – 19	46 (67.7)	27 (39.7)	17 (25.0)	6 (8.8)
20 – 29	61 (73.5)	36 (43.4)	11 (13.3)	7 (8.4)
30 – 39	43 (57.3)	27 (36.0)	9 (12.0)	2 (2.7)
40 – 49	31 (55.4)	18 (32.1)	6 (10.7)	1 (1.8)
≥50	29 (46.8)	20 (32.3)	4 (6.5)	0 (0.0)
p-value	<0.001			<0.001
Residence				
Rural	243 (64.5)	139 (36.9)	55 (14.6)	36 (9.6)
Urban	199 (60.3)	115 (34.9)	36 (10.9)	8 (2.4)
p-value	0.255			<0.001
Ethnic groups				
Nilo–Hamite	89 (55.6)	56 (35.0)	14 (8.8)	5 (3.1)
Bantu	195 (62.5)	114 (36.5)	43 (13.8)	18 (5.8)
Nilotic	104 (62.7)	62 (37.4)	20 (12.1)	9 (5.4)
Sudanese	54 (78.3)	22 (31.9)	14 (20.3)	12 (17.4)
p-value	0.014			0.001
Altitude				
≥1000 m	199 (60.3)	115 (34.9)	36 (10.9)	8 (2.4)
<1000 m	243 (64.5)	139 (36.9)	55 (14.6)	36 (9.6)
p-value	0.255			<0.001

* POC–CCA (point-of-care circulating cathodic antigen) test alone; Infection intensity only for Kato–Katz positive result (at least one S. mansoni egg in at least 1 of 4 smears).

Table S5.2i: *Schistosoma mansoni* infection prevalence (%) in the 2017 in–depth study. Study conducted in 12 purposively selected villages in Ituri province (n=707). Study participants provided two stool samples. From each sample, one to two Kato–Katz (KK) smears were examined (total two to four smears per person). In addition, each participant provided a urine sample for point–of–care circulating cathodic antigen (POC–CCA) test. POC-CCA results. Prevalence of the three diagnostic approaches by sex.

Variable	Female's prevalence (%)			Male's prevalence (%)		
	2KK+CCA	CCA	2KK	2KK+CCA	CCA	2KK
Overall	72.4	62.6	53.2	74.1	62.5	57.6
Age group (years)						
1 – 4	62.1	58.6	24.1	62.5	50.0	31.3
5 – 9	61.0	50.7	50.7	72.8	61.7	56.8
10 – 14	84.9	75.3	60.3	87.3	77.5	73.2
15 – 19	75.0	67.5	67.5	89.3	67.9	82.1
20 – 29	80.6	68.7	65.7	93.8	93.8	62.5
30 – 39	67.4	61.2	44.9	65.4	50.0	61.5
40 – 49	68.8	56.3	50.0	58.3	54.2	37.5
≥50	71.0	54.8	38.7	54.8	38.7	38.7
p -value	0.030	0.077	0.002	0.001	0.001	<0.001
Residence						
Rural	73.6	64.4	57.9	77.0	64.6	65.2
Urban	70.9	60.4	47.3	71.0	60.1	49.3
p -value	0.544	0.422	0.034	0.223	0.419	0.005
Ethnic group						
Nilo-Hamite	67.4	56.1	46.9	71.0	54.8	46.8
Bantu	74.3	63.1	54.2	72.2	61.7	58.7
Nilotic	69.3	63.4	52.3	74.4	64.1	57.7
Sudanese	84.9	81.8	66.7	86.1	75.0	72.2
p -value	0.208	0.071	0.257	0.350	0.253	0.103
Altitude						
≥1000 m	70.9	60.4	47.3	71.0	60.1	49.3
<1000 m	73.6	64.4	57.9	77.0	64.6	65.2
p -value	0.544	0.422	0.034	0.223	0.419	0.005

Table S5.3a. Demographic and socioeconomic risk factors for *Schistosoma mansoni* infection (2017 in–depth study). Results obtained with the univariate analysis of risk groups for infection with *S. mansoni* among participants from 12 villages in Ituri province in 2017 (n=707).

Risk factors	Infected n=517 (%)	OR (95% CI)	χ^2	p
Demographics				
Gender*				
Female	288 (72.4)	1.0		
Male	229 (74.1)	1.09 (0.78–1.53)	0.27	0.603
Age categories (year)*				
1 – 4	38 (62.3)	1.0		
5 – 9	106 (67.1)	1.23 (0.67–2.29)	0.45	0.504
10 – 14	124 (86.1)	3.75 (1.81–7.78)	14.59	<0.001
15 – 19	55 (80.9)	2.56 (1.13–5.79)	5.48	0.019
20 – 29	69 (83.1)	2.98 (1.34–6.63)	7.94	0.005
30 – 39	50 (66.7)	1.21 (0.60–2.46)	0.28	0.597
40 – 49	36 (64.3)	1.09 (0.51–2.32)	0.05	0.824
≥50	39 (62.9)	1.03 (0.49–2.14)	0.00	0.945
Residence				
Urban	234 (70.9)	1.0		
Rural	283 (75.1)	1.24 (0.88–1.72)	1.55	0.214
Ethnic groups				
Nilo–Hamites	110 (68.8)	1.0		
Bantu	229 (73.4)	1.25 (0.82–1.91)	1.13	0.287
Nilotic	119 (71.7)	1.15 (0.72–1.85)	0.34	0.563
Sudanese	59 (85.5)	2.68 (1.25–5.75)	6.97	0.008
Body mass index				
Obese	37 (58.7)	1.0		
Overweight	18 (75.0)	2.11 (0.72–6.14)	1.96	0.162
Normal weight	204 (75.3)	2.14 (1.20–3.82)	6.94	0.008
Underweight	258 (73.9)	1.99 (1.14–3.49)	6.05	0.014
Year of residence*				
1 – 4	166 (67.8)	1.0		
5 – 9	180 (70.3)	1.13 (0.77–1.65)	0.38	0.536
≥10	171 (83.0)	2.32 (1.47–3.68)	13.76	<0.001
Socioeconomic status				
Housing*				
Good	23 (57.5)	1.0		
Not good	494 (74.1)	2.11 (1.10–4.06)	5.26	0.022
Shoes possession*				
Owning shoes	194 (68.3)	1.0		
Not owning shoes	323 (76.4)	1.50 (1.07–2.10)	5.59	0.018

*Included in multi variable logistic regression analysis.
n: number; OR: odds ratio; CI: confidence interval.

Table S5.3b. Environmental risk factors for *Schistosoma mansoni* infection (2017 in–depth study). Results of the univariate analysis of risk groups for infection with *S. mansoni* among participants from 12 villages in Ituri province (n=707).

Risk factors	Infected n=517 (%)	OR (95% CI)	χ^2	p
Geographic risk factors				
Health District*				
Nia–Nia	38 (52.7)	1.0		
Tchomia	49 (70.0)	2.09 (1.03–4.22)	4.41	0.036
Bunia	234 (70.9)	2.18 (1.29–3.69)	8.86	0.003
Angumu	87 (73.1)	2.43 (1.30–4.57)	8.16	0.004
Lolwa	71 (93.4)	12.71 (4.02–40.18)	31.26	<0.001
Mandima	38 (95.0)	17.00 (3.21–89.99)	20.83	<0.001
Region				
Central region	234 (70.9)	1.0		
Eastern region	136 (72.0)	1.05 (0.71–1.57)	0.06	0.800
Southwestern region	147 (78.2)	1.47 (0.97–2.24)	3.26	0.071
Environmental risk factors				
Elevation level				
High (≥1000 m)	234 (70.9)	1.0		
Low (<1000 m)	283 (75.1)	1.24 (0.89–1.72)	1.55	0.214
Vegetation				
Savanna	370 (71.3)	1.0		
Forest	147 (78.2)	1.44 (0.97–2.15)	3.34	0.068
Distance to the nearby water bodies*				
Far (≥500 m)	166 (64.8)	1.0		
Near (<500 m)	351 (77.8)	1.90 (1.35–2.68)	13.99	<0.001

*Included in multi variable logistic regression analysis.
n: number; OR: odds ratio; CI: confidence interval.

Table S5.3c. Behavioural risk factors for *Schistosoma mansoni* infection (2017 in–depth study). Results of the univariate analysis of risk groups for infection with *Schistosoma mansoni* among participants from 12 villages in Ituri province (n=707).

Risk factors	Infected n=517 (%)	OR (95% CI)	χ^2	p
Presence of latrine in the household*				
Yes	418 (70.9)	1.0		
No	99 (84.6)	2.26 (1.32–3.87)	9.40	0.002
Declare using a latrine				
Yes	420 (72.2)	1.0		
No	97 (77.6)	1.34 (0.84–2.11)	1.54	0.214
Use of soap for washing hands				
Yes	242 (71.4)	1.0		
No	275 (74.7)	1.19 (0.85–1.65)	1.00	0.317
Bathing in streams*				
No	212 (70.2)	1.0		
Yes	305 (75.3)	1.29 (0.93–1.81)	2.30	0.130
Bathing in the lake				
No	501 (72.9)	1.0		
Yes	16 (80.0)	1.49 (0.49–4.50)	0.49	0.482
Swimming in water bodies*				
No	452 (71.8)	1.0		
Yes	65 (84.4)	2.13 (1.12–4.06)	5.60	0.018
Washing clothing in water bodies*				
No	100 (65.4)	1.0		
Yes	417 (75.3)	1.61 (1.10–2.38)	5.98	0.014
Washing dishes in water bodies				
No	505 (72.9)	1.0		
Yes	12 (85.7)	2.23 (0.49–10.10)	1.15	0.284
Fishing*				
No	468 (72.1)	1.0		
Yes	49 (84.5)	2.11 (1.01–4.39)	4.14	0.042
Farming*				
No	211 (68.1)	1.0		
Yes	306 (77.1)	1.58 (1.13–2.21)	7.19	0.007
Trading				
No	490 (73.2)	1.0		
Yes	27 (71.1)	0.90 (0.44–1.85)	0.09	0.767
Cleaning motorcycles in water bodies				
No	498 (72.4)	1.0		
Yes	19 (100.0)	– –	7.17	0.007

*Included in multi variable logistic regression analysis.
n: number; OR: odds ratio; CI: confidence interval.

Table S5.3d. Family and individual risk factors for *Schistosoma mansoni* infection (2017 in–depth study). Results of the univariate analysis of risk groups for infection with *Schistosoma mansoni* among participants from 12 villages in Ituri province (n=707).

Risk factors	Infected n=517 (%)	OR (95% CI)	χ^2	p
Family history of schistosomiasis*				
No	433 (74.4)	1.0		
Yes	84 (67.2)	0.71 (0.46–1.07)	2.71	0.100
Prior treatment for schistosomiasis*				
No	431 (72.1)	1.0		
Yes	86 (78.9)	1.45 (0.88–2.38)	2.18	0.140
Knowledge of schistosomiasis as a disease				
No	382 (71.9)	1.0		
Yes	135 (76.7)	1.28 (0.86–1.91)	1.52	0.217
Knowledge of the transmission of schistosomiasis				
No	397 (72.5)	1.0		
Yes	120 (75.5)	1.17 (0.78–1.76)	0.57	0.449
Knowledge of the prevention of schistosomiasis*				
No	392 (71.5)	1.0		
Yes	125 (78.6)	1.46 (0.96–2.23)	3.14	0.076
Knowledge of praziquantel as treatment of schistosomiasis*				
No	482 (74.3)	1.0		
Yes	35 (60.3)	0.53 (0.30–0.92)	5.24	0.022

*Included in multi variable logistic regression analysis.
n: number; OR: odds ratio; CI: confidence interval.

Table S5.4. Risk factors for *Schistosoma mansoni* infection (2017 in–depth study). Results of the multivariable analysis of risk groups for infection with *Schistosoma mansoni* among participants from 12 villages in Ituri province (n=707).

Risk factors	OR (95% CI)	Std. Err.	z	p–value
Demographic risk factors				
Age groups	0.92 (0.83–1.02)	0.049	–1.53	0.127
Gender (Male/Female)	1.20 (0.82–1.76)	0.234	0.94	0.348
Socioeconomic risk factors				
Poor housing (Yes/No)	2.10 (1.02–4.35)	0.779	2.01	0.044
Lack of shoes (Yes/No)	1.28 (0.86–1.91)	0.260	1.21	0.226
Environmental risk factor				
Health district	1.13 (1.04–1.23)	0.049	2.83	0.005
Water (<500m/≥500m)	1.72 (1.18–2.49)	0.325	2.85	0.004
Year of residence (≥10/<10)	1.41 (1.11–1.79)	0.174	2.78	0.005
Behavioural risk factors				
Lack of latrine (Yes/No)	2.00 (1.11–3.60)	0.602	2.30	0.022
Bathing (Yes/No)	0.88 (0.60–1.30)	0.175	–0.62	0.537
Swimming (Yes/No)	2.53 (1.20–5.32)	0.959	2.45	0.014
Washing (Yes/No)	1.75 (1.10–2.78)	0.412	2.37	0.018
Fishing (Yes/No)	1.95 (0.85–4.48)	0.826	1.58	0.115
Farming (Yes/No)	1.43 (0.94–2.18)	0.306	1.66	0.096
Knowledge				
Family history (Yes/No)	0.52 (0.29–0.94)	0.157	–2.17	0.030
Prior treatment (Yes/No)	1.63 (0.90–2.95)	0.493	1.61	0.108
Know prevention (Yes/No)	2.35 (1.36–4.08)	0.660	3.05	0.002
Know praziquantel (Yes/No)	0.33 (0.16–0.69)	0.125	–2.93	0.003

OR: odds ratio; CI: confidence interval.

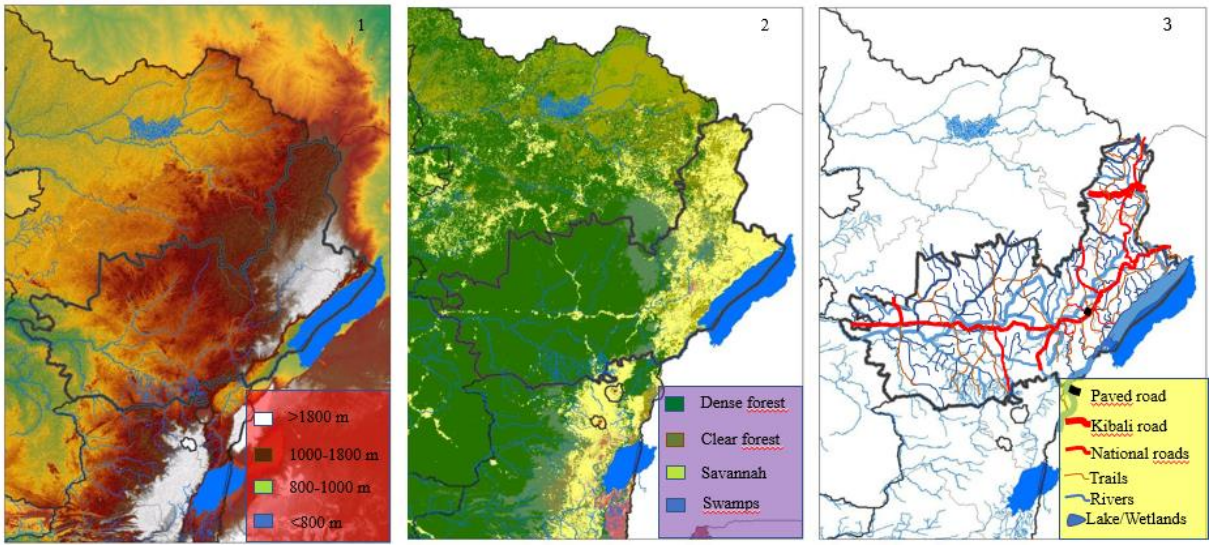

Figure S5.1: Geographic characteristics of Ituri province: This figure shows the study site : 1) Great geographic variability: high altitude up to 2,300 m above sea level and lowlands at about 500 m above sea level, large plateau and wide plains 2) Vegetation: dense forest in the southwestern region and grassy savanna along the eastern part 3) Water distribution and roads: a heavily irrigated region with several rivers, streams, swamps, ponds, and Lake Albert. There are very few roads made of laterite – the only passable road during the year is that which leads to Kibali gold mine in the neighbouring province of Haut–Uélé; only 5km of road are paved throughout the province, at Bunia city.

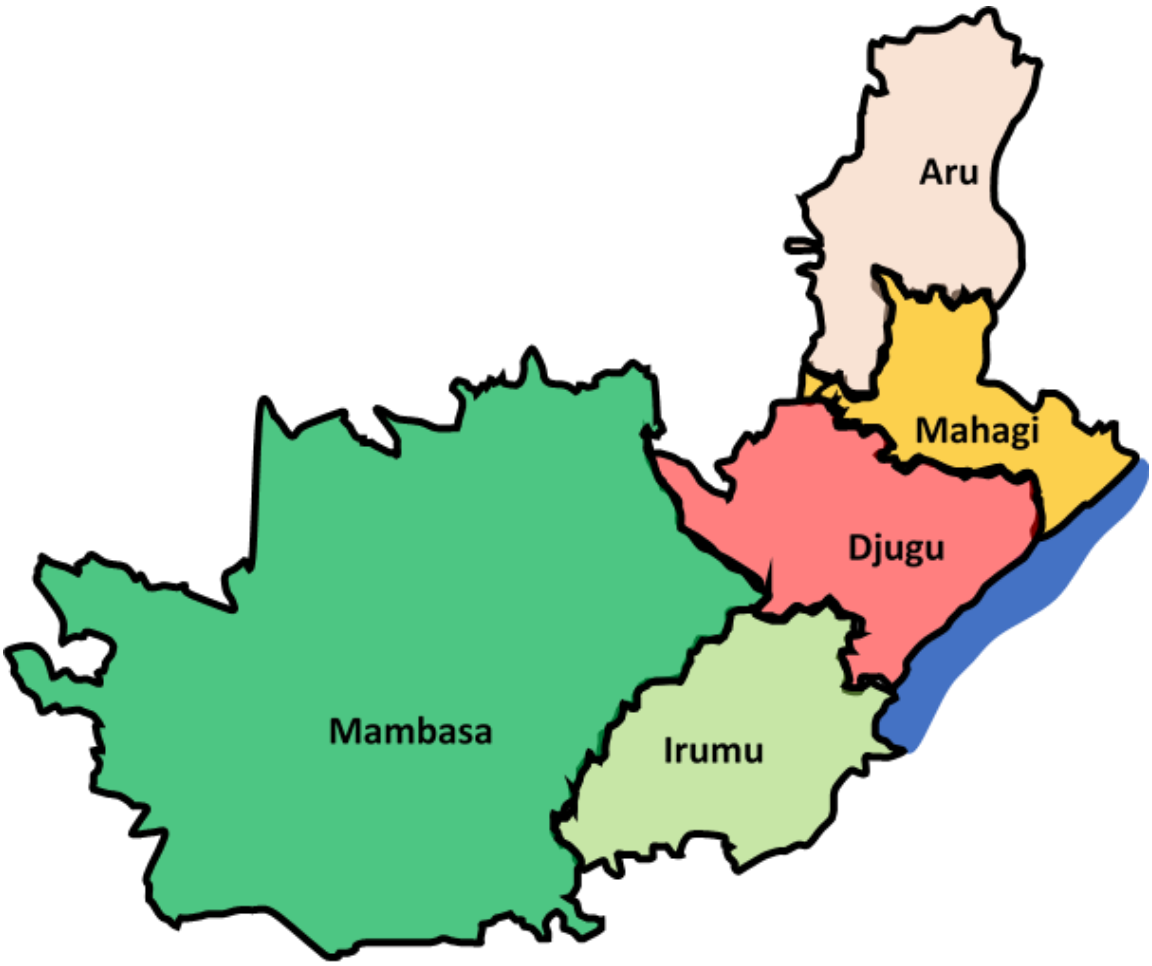

Figure S5.2a: Administrative subdivision of Ituri province: Ituri province is divided into 1) 5 administrative counties (or "territoires"): Mambasa (36,785 km²), Irumu (8,183 km²), Djugu (8,730 km²), Mahagi (5,216 km²), and Aru (6,749 km²); only 9% of Ituri's population live in the largely forest–covered and reserve territory of Mambasa [307].

Figure S5.2b: Health districts in Ituri province: Ituri province is divided into 36 health districts: 14 were visited (brownish colour) during our two surveys in 2016 and 2017, including Adi, Laybo, Logo, Nyarambe, Angumu, Rethy, Bambu, Bunia, Tchomia, Nyankunde, Komanda, Lolwa, Mandima, and Nia–Nia [307].

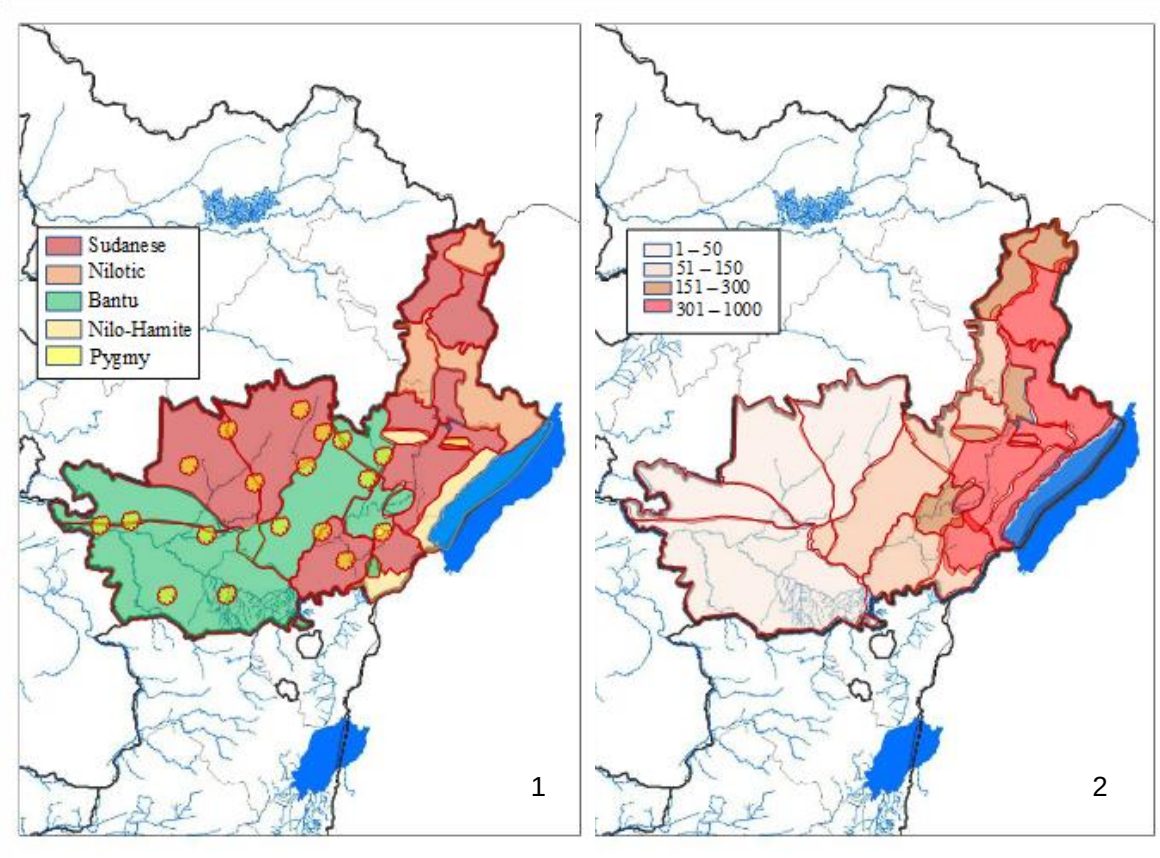

Figure S5.3: Ethnic composition and population density in Ituri province: this figure shows hat 1) Ituri province is populated by more than 40 tribal groups, belonging to one of five ethnic groups: Sudanese, Nilotic, Bantu, Nilo–Hamite, and Pygmy; 2) The mean density is about 80.8 inhabitants/km². However, around 91.7% of the inhabitants live in 44.0% of the area, in the eastern half of the province, and only 8.3% live in 56.0% of the area, in the southwestern half of the province covered by the forest reserve [307].

6 Morbidity associated with *Schistosoma mansoni* in northeastern Democratic Republic of the Congo

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Short title: Morbidity associated with schistosomiasis in Ituri province in Northeastern Congo

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This article is in revision in the *PLoS Neglected Tropical Diseases journal*

6.1 Abstract

Background

Controlling morbidity is the main target of schistosomiasis control. Yet, only rarely do they assess morbidity linked to *Schistosoma* sp. infection. In the Democratic Republic of Congo (DRC), and particularly in the north-eastern Ituri province, morbidity associated with *Schistosoma mansoni* infection is unknown. For this reason, we aimed to assess intestinal and hepatosplenic morbidity associated with *S. mansoni* infection in Ituri province.

Methods / Principal Findings

In 2017, we conducted a cross-sectional study in 13 villages in Ituri province, DRC. *S. mansoni* infection was assessed with a Kato-Katz stool test (2 smears) and a point-of-care circulating cathodic antigen (POC-CCA) test in urine. A questionnaire was used to obtain demographic data and information about experienced intestinal morbidity. Each participant underwent an abdominal ultrasonography examination to diagnose hepatosplenic morbidity. Of the 586 study participants, 76.6% tested positive for *S. mansoni*. Intestinal morbidity, such as abdominal pain (52.7%), diarrhoea (23.4%) and blood in the stool (21.5%) in the previous two weeks, was very frequent. Hepatosplenic morbidity was revealed by abnormal liver parenchyma patterns (42.8%), hepatomegaly (26.5%), and splenomegaly (25.3%). Liver pathology (adjusted odds ratio [aOR] 1.20, 95% confidence interval [CI] 1.06–1.37, P=0.005) was positively and significantly associated with *S. mansoni* infection. Hepatomegaly (aOR 1.52, 95% confidence interval [CI] 0.99–2.32, P=0.053) and splenomegaly (aOR 1.12, 95% CI 0.73–1.72, P=0.619) were positively but not significantly associated with *S. mansoni* infection at the individual level. At the village level, *S. mansoni* prevalence was positively associated with the prevalence of hepatomegaly and splenomegaly. Higher *S. mansoni* infection intensities were associated with diarrhoea, blood in the stool, hepatomegaly, splenomegaly, and liver parenchyma (pathology patterns C, D, E and F). Four study participants were diagnosed with ascites and five reported hematemesis.

Conclusions/Significance: Our study documents a high burden of intestinal and hepatosplenic morbidity associated with *S. mansoni* infection status in Ituri province. The results call for targeted interventions to address both *S. mansoni* infection and related morbidity.

Keywords: *Schistosoma mansoni;* Ituri province; Democratic Republic of Congo; infection prevalence; infection intensity; hepatosplenic morbidity.

6.2 Author Summary

Schistosomiasis caused by *Schistosoma mansoni* is of great public health importance in sub-Saharan Africa. The World Health Organization (WHO) recommends that control efforts aim to reduce morbidity through large scale intervention programmes. However, intestinal and liver morbidity is rarely assessed in such control programmes. Hence, little is known about (i) the magnitude of the intestinal and liver morbidity burden in each community, or about (ii) the morbidity associated with *S. mansoni* infection, specifically. We conducted a (cross-sectional) study in which we assessed intestinal morbidity by questionnaire and liver morbidity by abdominal ultrasonography. Further, we determined the infection status of the study participants using standard diagnostic procedures (Kato-Katz technique and point-of-care cathodic circulating *S. mansoni* antigen [POC-CCA] test in urine). Among 586 study participants, six years and older, from 13 villages in Ituri province, DRC, we observed a high degree of intestinal (e.g. 23.4% with diarrhoea; 21.5% with blood in stool) and hepatosplenic morbidity (e.g. 42.8% with abnormal liver patterns C, D, E, and F; 26.5% with an enlarged liver; 25.3% with an enlarged spleen). *S. mansoni* infection was associated with liver and spleen enlargement. Likewise, *S. mansoni* infection intensity was linked to diarrhoea, to liver and spleen enlargement and to pathological changes in the liver parenchyma. At village level, we observed that the prevalence of enlarged liver and spleen among patients increased with the prevalence of *S. mansoni* infection. We conclude that the population of Ituri province carries an alarming burden of intestinal, liver and spleen morbidity associated with *S. mansoni* infection. Therefore, a comprehensive control programme to address this infection and disease burden is urgently required.

6.3 Introduction

Schistosomiasis is a chronic helminth infection caused by trematodes of the genus *Schistosoma* and one of the so-called neglected tropical diseases. It is a major cause of morbidity and mortality around the globe [288].

Depending on the species, the disease may be genitourinary (*Schistosoma haematobium*) or intestinal (*Schistosoma mansoni, S. japonicum, S. mekongi, S. intercalatum,* and *S. guineensis*). The disease arises from the host's cell-mediated granulomatous immune response to the soluble antigens of the parasite eggs trapped in the tissues [52, 285]. In the intestinal form, the adult worm dwells in the portal vein and mesenteric veinlets that drain the intestines, where the female deposits her eggs during her daily migration. Chronic and heavy infections are frequently associated with hepatosplenic and intestinal diseases, characterized by liver and spleen enlargements and intestinal damage. In the liver, the resulting scars may disrupt liver function and obstruct the portal veins, leading to periportal fibrosis (PPF), portal hypertension, and subsequently to oesophageal varices, hematemesis and melena, and ultimately to ascites, the main cause of death due to *S. mansoni* infection [30, 74]. In the intestines, inflammation may induce diarrhoea, while granulomas may cause polyposis with ulcers and recurrent bleeding. The resulting clinical manifestations may include abdominal pain, diarrhoea, and the presence of blood in the stool [285, 334]. Chronic schistosomiasis can also lead to anaemia, stunted growth and impairment of cognitive development [65, 66]. Many infected people, even those with considerable infection intensity, may remain asymptomatic for a long period or experience only non-specific symptoms, such as nausea, headaches, fever, fatigue and abdominal pain [44].

Preventive chemotherapy (PCT) through mass drug administration (MDA) is the WHO-recommended strategy for both reducing morbidity and controlling schistosomiasis in endemic settings [286]. However, PCT is poorly implemented in many countries, often because of a lack of commitment or funding, and/or because of political instabilities and security issues, among other factors.

In the Democratic Republic of Congo (DRC), the full extent of the schistosomiasis morbidity burden remains unknown; relevant information is more than twenty years old [13, 123]. Existing publications report on *Schistosoma* infection. The few reports related to morbidity mainly concern

the province of Maniema, in the central-eastern region of the country [299, 335]. Morbidity due to *S. mansoni* infection in Ituri province was mentioned in colonial times [118]. Since then, only these data and those from the 1970s and 1980s have been summarized in the available reviews. Madinga et al. [123] reported that *S. mansoni* endemic areas in Ituri were described before 1954, with prevalence rates ranging from 11.0% to 64.9% along the left bank of Lake Albert. Conversely, Gillet and Wolfs reported an absence of local cases in the high hill region, and that prevalence ranged from 2.3% in Aru, in the north, to 93.7% in a fishing village on the shore of Lake Albert [118]. Neither review mentioned the existence of *S. haematobium* and *S. intercalatum* infections in Ituri province [123, 312]. By investigating the prevalence, intensity, and relative morbidity of *S. mansoni* infection among Ugandan and Zairian school children, aged 5 to 20 years, in Aru region, Müller et al. found that prevalence was low to moderately high. About 8.0% of children had heavy infections. Among the children, 15.6% to 38.0% had hepatomegaly, while 22.0% to 59.2% were diagnosed with splenomegaly. However, organomegaly associated with *S. mansoni* infection was not found to be significant [131].

To the best of our knowledge, the only recent study undertaken since the 1980s was the national survey conducted between 2013 and 2015. The survey results have not yet been published. However, several studies conducted in neighbouring Uganda show high *S. mansoni* infection and morbidity rates, and considerable mortality linked to infection [30, 52, 336]. The aim of the present study was to assess the morbidity associated with *S. mansoni* infection in Ituri province, DRC.

6.4 Materials and Methods

Ethics statement

This study was approved by the Swiss Ethical Commission (Ref. No. UBE-15/78) and by the University of Kisangani's Research Ethical Commission, (Ref No: CER/003/GEAK/2016). Research authorization was granted by the Nyankunde Higher Institute of Medical Techniques (Ref No 70/ISTM-N/SGAC/2017), Bunia, DRC. Permission for field work was obtained from the Ituri Provincial Health Division (Ref. 054/433/DPS/IT/06/2016 and Ref. 054/472/DPS/IT/06/2017) and from all relevant health districts. Prior to enrolment, the study objectives and procedures were explained to each participant in the local language and all their questions were answered. Written informed consent was obtained from all study participants aged 15 years and older. Parents or legal guardians signed consent forms for participants aged <14 years. Participants diagnosed with *S. mansoni* were treated with praziquantel (40mg/kg) [141]. All participants received Mebendazole (500mg, single dose, Vermox®) for general deworming, in accordance with the DRC national deworming guidelines.

Study area

The study was conducted in Ituri province, north-eastern DRC (geographical coordinates: 1.30°–3.60° latitude and 27.00°–31.40° longitude). Ituri province has an area of about 65,658 km^2 and is home to 5.2 million inhabitants from five different ethnic groups (Nilo-Hamites, Bantu, Nilotic, Sudanese, and Pygmy). The province is divided into five territories (counties) and 36 health districts, and is bordered by Lake Albert in the east, while several streams and rivers irrigate the province. These waterways are suitable environments for schistosome's intermediate host snails. For this study, six health districts were purposively selected because of their high prevalence of *S. mansoni* infection: Angumu, Bunia, Lolwa, Mandima, Nia-Nia, and Tchomia. From these health districts, a total of 13 villages were purposively selected. From the biggest health district, Bunia, with a population of more than 500,000 inhabitants, five villages were selected (Lumumba, Simbilyabo, Kindia, Gupe, Sukisa, and Ngezi). From the other health districts, two villages were selected from Angumu (Gupe and Ndaru-Muswa), two from Lolwa (Mambau and Pekele), two from Nia-Nia (Bankoko and Mangenengene), one from Mandima (Mandima), and one from Tchomia (Kadjugi). The presence of *Schistosoma mansoni* in the province was widely documented

during colonial times, with transmission thought to occur mainly along Lake Albert's shores [118]. Neither a review of the available literature nor a consultation with the provincial NTD control programme suggested the presence of *S. haematobium* in Ituri province, nor did we find *S. haematobium* during our prior work in the area (MNN, unpublished information). *Schistosoma intercalatum*, however, is mentioned by a few authors. For these reasons, we concentrated our efforts on studying morbidity related to intestinal schistosomiasis (*S. mansoni* and *S. intercalatum*) in Ituri province [118, 123, 312]. Only a small proportion of the population residing in Bunia city has access to an adequate water supply. Most of the population uses natural water bodies (springs, ponds, streams) as its main water source.

Study design and population

We conducted a cross-sectional, household-based, in-depth study of the 13 purposely selected villages across six health districts in Ituri province. Two-stage sampling procedures were used to select both households and individuals for the study. At least 10 households were randomly selected in each village, and all individuals aged six years and older and present on the day of the survey were enrolled. Household visitors, as well as mentally and terminally ill persons were excluded.

The study incorporated household and individual questionnaires; anthropometric assessments; and parasitological, clinical, and abdominal ultrasonographic examinations.

Procedures

Individual questionnaires

All participants were invited to participate in an interview, conducted using a pre-tested questionnaire. This individual questionnaire focused on demographic, anthropometric, occupational, educational, and religious characteristics, as well as on knowledge, attitude and practices related to *S. mansoni* infection and disease. The questionnaire also helped to assess for signs and symptoms related to schistosomiasis, such as diarrhoea or blood in the stool in the previous two weeks, and a history of hematemesis at any time, at least once.

Anthropometric measurements

Participants' height and weight were measured by a Seca analog bathroom scale and height rod and reported to the nearest half kilogram (0.5 kg) and half centimetre (0.5 cm), respectively. Participants' body mass index (BMI) was also calculated (weight in kilograms divided by the square of the person's height in metres, kg/m^2).

Parasitological examination

Participants were asked to provide one faecal sample (approx. 5 grams of morning stool) in a labelled plastic container for testing with the Kato-Katz technique [79]. From each stool specimen, two thick smears of 41.7 mg [79] were prepared and examined by experienced technicians. To allow for hookworm assessment, all smears were examined by microscope within one hour after preparation. All slides were examined for *S. mansoni* within 24 hours after stool collection. One third of the prepared smears were checked by the principal investigator. All helminth eggs were counted and recorded for each species separately. The intensity of the helminth infection was calculated by multiplying the mean number of eggs found on the two slides by 24. The result was expressed as eggs per gram (EPG) of stool [66].

Participants were also asked to provide a urine sample (approx. 60 ml) in a pre-labelled, wide-mouth, plastic container, for the detection of circulating *S. mansoni* antigens using a point-of-care circulating cathodic antigen (POC-CCA) test. Both the stool and urine examinations were performed at the relevant village health centre facility.

The POC-CCA tests were performed according to the manufacturer's guidelines (Rapid Medical Diagnostics, Pretoria, South Africa). Urine was examined on the day of collection. In cases where the test was postponed until the next day, urine samples were kept in a solar fridge, at 2–8ºC (Steca, Germany). Test results were deemed negative if the POC-CCA band did not appear within 20 minutes. Trace, weak, medium, and strong coloured CCA bands were recorded as positive results. Questionable results were discussed among at least two technicians and the principal investigator.

Clinical examination

All participants underwent clinical and abdominal ultrasonography examinations. Clinical examinations consisted of physical examinations performed by an experienced physician and assisted by an experienced nurse.

Abdominal ultrasound examination

An abdominal ultrasound was performed for each participant, in accordance with the Niamey protocol [55, 337] and using a 2.0 MHz convex transducer U-Lite Sonoscanner Ultraportable HD Ultrasound Unit (U-Lite, Sonoscanner, 6, Rue André Voguet, Paris, France). A portable generator (MK, China) and solar powered batteries (for remote villages) were used as electricity sources.

The size of the left lobe, from the cranial to the caudal edge of the liver, was measured at about two centimeters from the xyphoid, in the left parasternal line (PSL). The length and width of the spleen were also measured, and its texture evaluated. All measures were taken in centimetres and performed using the callipers according the manufacturer's recommendations. Organ measurements were then adjusted to the height of the individual and compared with those of the healthy Senegalese control group [338]. Enlarged organs were defined as those with a size exceeding two standard deviations (2 SD) from the mean adjusted values for individuals in the control group. Liver parenchyma patterns (Figure S1) were assessed following the WHO/TDR guidelines as follows: grade A, normal; grade B, incipient; grade C, probable; grades D, E, and F, frank periportal fibrosis [55, 337]. The diameter of the inner portal vein was measured. The length, width, and wall thickness of the inner gall bladder were also measured. Other liver patterns that are not linked with schistosomiasis such as fatty-liver-like (pattern Y), and other abnormalities (pattern Z) [337], were considered separately.

Data management and analysis

Data was entered in Excel and cross-checked against the data sheet. STATA, version14.2 software (Stata Corp, College Station, USA) was used for data management and analysis. Only participants with a complete dataset were retained in the analysis (Figure 1). Seven age groups were established: (i) 6–9 years, (ii) 10–14 years, (iii) 15–19 years, (iv) 20–29 years, (v) 30–39 years, (vi) 40–49 years, and ≥50 years. Body mass index (BMI) was calculated and four categories were set:

underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30 kg/m²). Infection prevalence was expressed as the number of *S. mansoni*-positive individuals divided by the total number of participants examined. Infection intensity was estimated based on helminth egg counts per gram of stool (EPG) when examined with the Kato-Katz technique [79]. *S. mansoni* infection intensities were classified as light (1–99 EPG), moderate (100–399 EPG), and heavy (≥400 EPG) [66].

Arithmetic mean infection intensity was calculated. Categorical variables were presented as frequencies and percentages. Pearson's chi-square (χ^2) test was used to compare frequency distributions. A univariate logistic regression analysis was carried out to identify associations between *S. mansoni* infection status (outcome) and morbidity indicators (predictors) and/or demographic factors (age, gender). Predictors with a significance level of 20% or less, and age and gender variables were included in the multivariable logistic regression models. Odds ratios (OR), adjusted OR (aOR), and corresponding 95% confidence intervals (95% CI) were calculated. *P*-values <0.05 were considered statistically significant. In this study, we combined the results of the Kato-Katz technique and those of the POC-CCA to diagnose disease status. For comparison, the same analysis was repeated for both the Kato-Katz and the POC-CCA diagnostic approaches.

Ultrasonographic organ measures were defined as enlarged if the left liver lobe and/or spleen length exceeded the normal reference value by 2 SD. Likewise, portal vein diameter was considered enlarged if it exceeded the normal reference value by 2 SD. Liver patterns A and B were considered normal, patterns C and D were deemed mild PPF, and patterns E and F were recorded as severe PPF. Liver patterns Y and Z were included in the analysis but neither as PPF nor normal patterns [337].

6.5 Results

Study population

Data were collected between June and September 2017. We enrolled participants from 13 purposely selected villages across six health districts with an anticipated high prevalence of *S. mansoni* infection. Of the 949 individuals enrolled (Figure 6.1), 586 completed all study procedures and had a complete dataset, that is, one stool sample examined with two Kato-Katz smears, a urine sample tested with POC-CCA, two completed questionnaires, and a clinical and abdominal ultrasound examination.

Among those with a complete dataset, 342 (58.4%) were females, 330 (56.3%) were under 20 years of age, and 268 (45.7%) were underweight (Table 6.1). The prevalence of *S. mansoni* was 59.2%, 65.7%, and 76.6% according to Kato-Katz, POC-CCA and combined test results, respectively. Thirty-seven percent, 15.2% and 7.2% of the population had light-, moderate- and heavy-intensity infections, respectively. Infection with soil transmitted helminths (STH) was not common among participants, with only eight participants diagnosed with an STH infection. In contrast, intestinal symptoms were very common, with 52.7%, 23.4% and 21.5%, reporting abdominal pain, diarrhoea, and blood in the stool within the two weeks preceding the survey, respectively. Five participants (0.9%) had experienced hematemesis at least once in his/her life. Abdominal ultrasound examinations revealed that 26.5% of participants had hepatomegaly, 25.3% had splenomegaly, and 42.8% had liver pathology; 36.4% had mild PPF (patterns C and D), 6.4% had severe PPF (patterns E and F), and 1.0% presented fatty liver pattern patterns Y, 0.2% other non-identified abnormality (pattern Z) not linked with schistosomiasis, and 0.7% had ascites. Only 56.0% of the participants had a normal liver parenchyma (patterns A and B). More details on liver parenchyma patterns are shown in Tables S6.7 and S6.8.

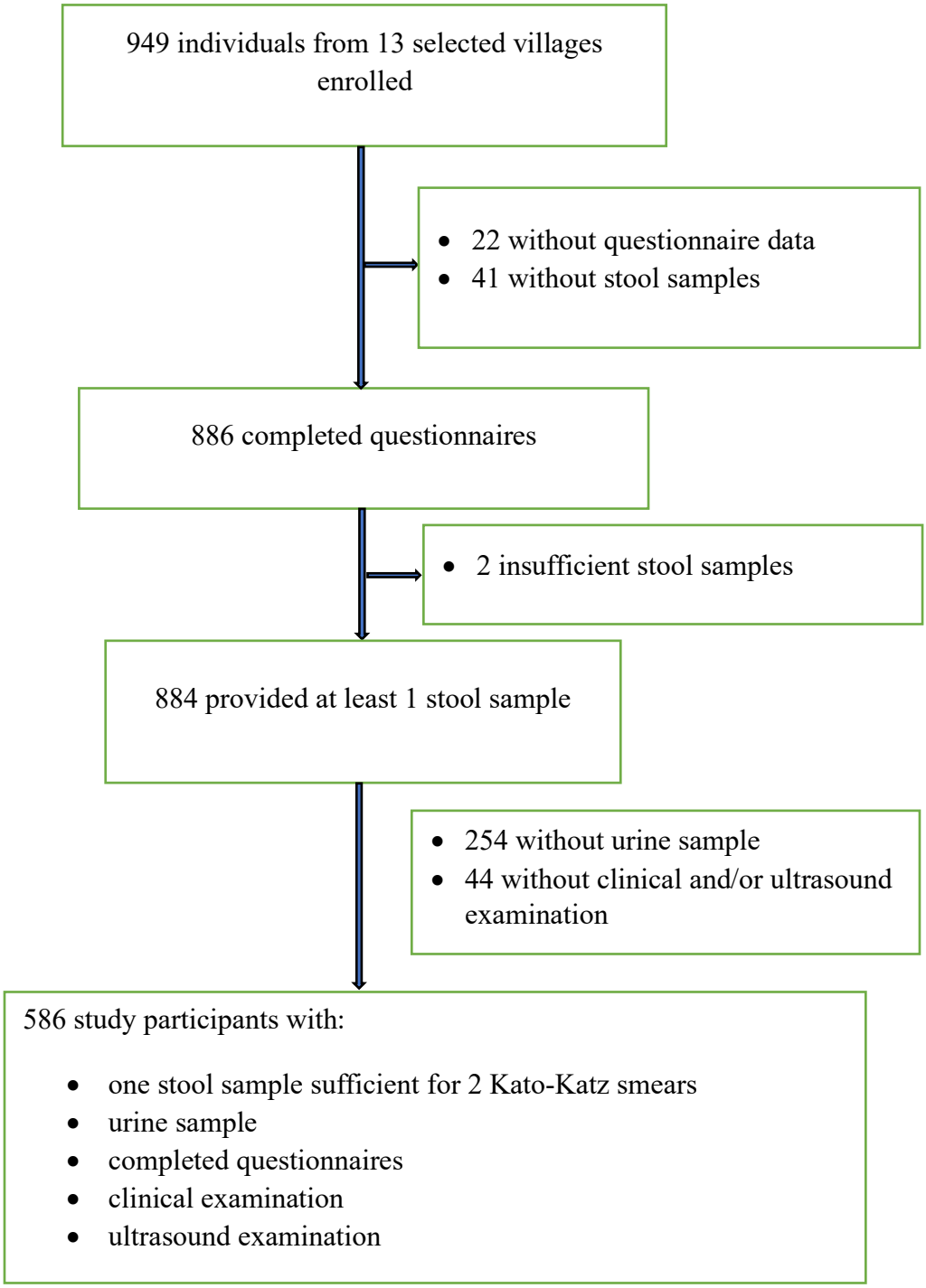

Figure 6.1: Flowchart of participant inclusion/exclusion in the 2017 Ituri morbidity study across 13 villages.

Table 6.1: Study sample characteristics in the 2017 Ituri infection and morbidity study.
Study conducted in 13 purposively selected villages in Ituri province (n=586).

Characteristics	N	%
Gender Females	342	58.4
Males	244	41.6
Age categories (years)		
6 – 9	123	21.0
10 – 14	140	23.9
15 – 19	67	11.4
20 – 29	77	13.1
30 – 39	68	11.6
40 – 49	52	8.9
≥50	59	10.1
Body mass index (kg/m² - categories)		
Obese (≥30.0)	58	9.9
Overweight (25.0–29.9)	24	4.1
Normal weight (18.5–24.9)	236	40.3
Underweight (<18.5)	268	45.7
S. mansoni infection		
Kato-Katz test	347	59.2
CCA test	385	65.7
KK+CCA*	449	76.6
Infection intensity (KK only)		
Light	216	36.9
Moderate	89	15.2
Heavy	42	7.2
Soil transmitted helminths		
Trichuris trichiura	3	0.5
Ascaris lumbricoides	1	0.2
Hookworm	4	0.7
Clinical findings		
Diarrhoea	137	23.4
Blood in stool	126	21.5
Abdominal pain	309	52.7
Hematemesis	5	0.9
Ultrasound findings		
Hepatomegaly (US)	155	26.5
Splenomegaly (US)	148	25.3
Ascites	4	0.7
Patterns A/B	328	56.0
Patterns C/D	213	36.4
Patterns E/F	38	6.4
Fatty liver	6	1.0
Other abnormality	1	0.2

* KK+CCA, combined any positive result by Kato-Katz and/or by point-of-care circulating cathodic antigen (POC-CCA); KK only, Kato-Katz results only with at least one egg in at least one of two smears.

Morbidity associated with *S. mansoni* infection

The results of the univariable risk analysis of the combined approach are presented in Table 6.2. Male participants were more likely to be infected with *S. mansoni,* but the increased risk was not statistically significant (OR 1.22, 95% CI 0.82–1.81, *P*=0.318). *S. mansoni* infection was observed more frequently in younger age groups, with prevalence peaking among young adults (Figure 6.3). Participants aged 50 years and older had a statistically significant reduced risk of infection compared to children aged 6–9 years (Table 2, OR 0.49, 95% CI 0.26–0.92, *P*=0.024).

Intestinal helminth co-infections were negatively associated with *S. mansoni* infection status; of these, the association with hookworm infection was statistically significant (OR 0.10, 95% CI 0.01–0.98, P=0.015). Study participants who reported an episode of diarrhoea within the preceding two weeks had an increased risk of *S. mansoni* infection (OR 1.69, 95% CI 1.03–2.78, *P*=0.038).

Diagnosed hepatomegaly (OR 1.38, 95% CI 0.87–2.17, *P*=0.168), splenomegaly (OR 1.27, 95% CI 0.81–2.01, P=0.302) and E/F liver parenchyma patterns (OR 1.25, 95% CI 0.55–2.84, *P*=0.593) were positively but not significantly associated with an *S. mansoni* infection.

The univariable risk analyses of the Kato-Katz and POC-CCA diagnostic approaches are given in supplementary Tables S6.1 and S6.3, respectively, and show a very similar risk pattern. It is worth noting that when the results of the Kato-Katz tests were considered, male participants had a significantly higher risk of *S. mansoni* infection (Table S6.1, OR 1.44, 95% CI 1.03–2.03, *P*=0.033); and, unlike splenomegaly (Table S1, OR 1.61, 95% CI 1.09–2.39, *P*=0.017), hepatomegaly (Table S6.1, OR 1.41, 95% CI 0.96–2.06, *P*=0.079) was not significantly associated with *S. mansoni* infection.

Table 6.2: Morbidity associated with *S. mansoni* infection in the 2017 study. Results of the univariable analysis of data from 13 purposively selected villages in Ituri province (n=586).

Characteristics	*S. mansoni* (+) N=449		*S. mansoni* (-) N=137			
	n	%	n	%	OR (95% CI)	p-value
Gender*						
Females	257	57.2	85	62.0	1.0	
Males	192	42.8	52	38.0	1.22 (0.82–1.81)	0.318
Age categories (years)*						
6 – 9	92	20.5	31	22.6	1.0	
10 – 14	119	26.5	21	15.3	1.61 (0.94–2.76)	0.078
15 – 19	54	12.0	13	9.5	1.10 (0.58–2.07)	0.769
20 – 29	63	14.0	14	10.2	1.53 (0.81–2.89)	0.184
30 – 39	48	10.7	20	14.6	0.77 (0.42–1.42)	0.396
40 – 49	35	7.8	17	12.4	0.73 (0.38–1.43)	0.359
≥50	38	8.5	21	15.3	0.49 (0.26–0.92)	0.024
STH						
T. trichiura (Y/N)	1	0.2	2	1.5	0.15 (0.01–1.69)	0.076
A. lumbricoides (Y/N)	1	0.2	0	0.0	na	
Hookworm (Y/N) *	4	0.2	3	2.2	0.10 (0.01–0.98)	0.015
Anthropometry (BMI)*						
Obese (Y/N)	36	8.0	22	16.1	1.0	
Overweight (Y/N)	18	4.0	6	4.4	1.83 (0.62–5.40)	0.264
Normal weight (Y/N)	183	40.8	53	38.7	2.11 (1.14–3.92)	0.016
Underweight (Y/N)	212	47.2	56	40.9	2.31 (1.25–4.28)	0.006
Clinical findings						
Diarrhoea (Y/N) *	114	25.4	23	16.8	1.69 (1.03–2.78)	0.038
Blood in stool (Y/N)	99	22.1	27	19.7	1.15 (0.72–1.86)	0.560
Abdominal pain (Y/N)	238	53.0	71	51.8	1.05 (0.72–1.54)	0.808
Hematemesis (Y/N)	4	0.9	1	0.7	1.22 (0.14–11.05)	0.858
Ultrasound findings						
Hepatomegaly (Y/N) *	125	27.8	30	21.9	1.38 (0.87–2.17)	0.168
Splenomegaly (Y/N) *	118	26.3	30	21.9	1.27 (0.81–2.01)	0.302
Ascites (Y/N) *	2	0.5	2	1.5	0.30 (0.04–2.17)	0.207
Pattern A/B (Y/N) *	246	54.8	82	59.9	1.0	
Pattern C/D (Y/N)	167	37.2	46	33.6	1.21 (0.80–1.83)	0.363
Pattern E/F (Y/N)	30	6.7	8	5.8	1.25 (0.55–2.84)	0.593
Fatty liver (Y/N)	5	1.1	1	0.7	2.00 (0.24–16.94)	0.696
Other (Y/N)	1	0.2	0	0	na	0.580

* Included in the multivariable analysis. BMI, body mass index; na, not applicable; Pattern A: normal; Pattern B: "starry sky"; Pattern C: "rings and pipe-stems"; Pattern D "highly echogenic ruff around portal bifurcation"; Pattern E "highly echogenic patches"; Pattern F: "highly echogenic bands and streaks – bird's claw"; Fatty liver (pattern Y) and other abnormality (pattern Z) indicate pathology different from periportal fibrosis [55, 337].

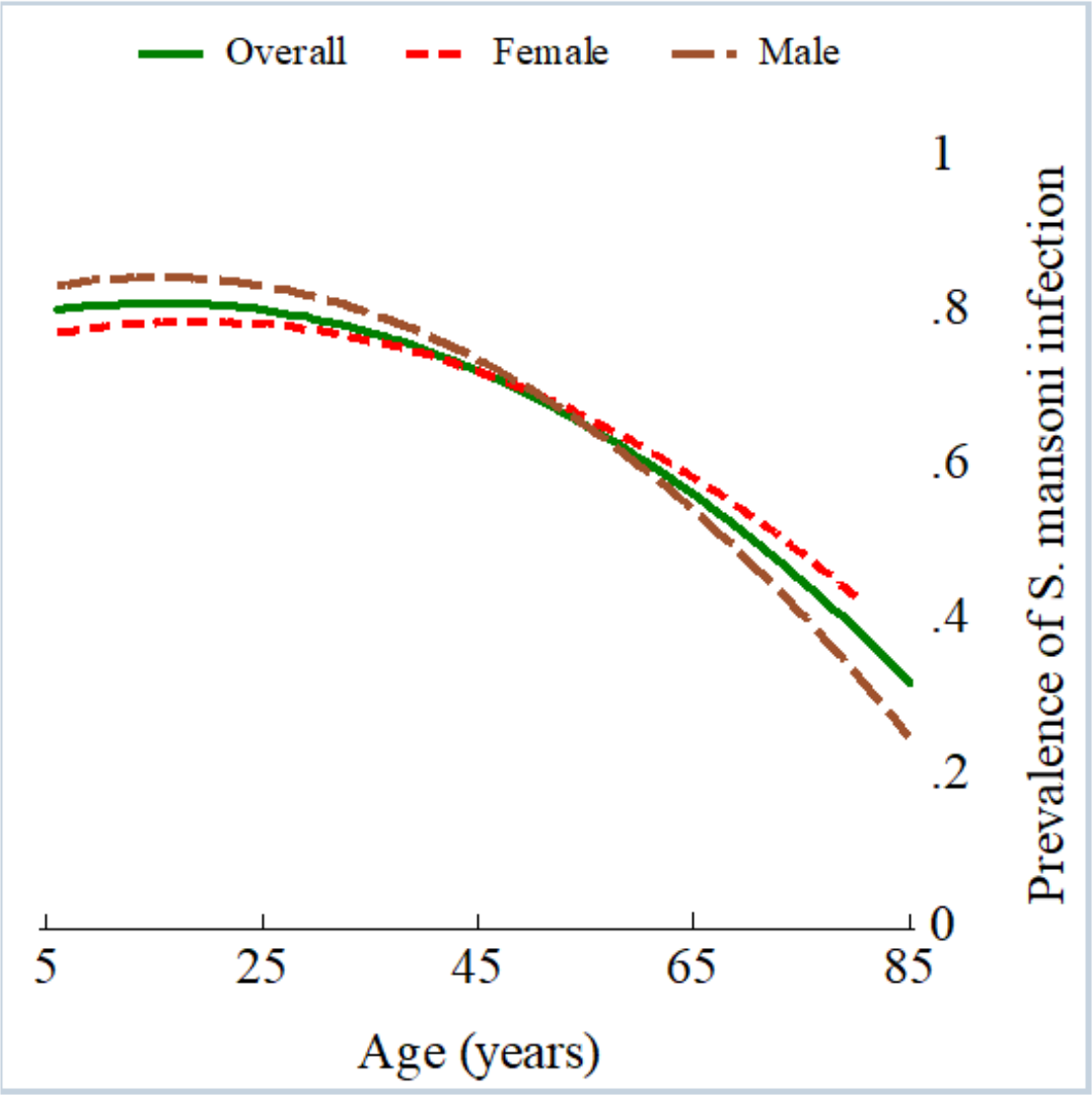

Figure 6.2: *S. mansoni* infection prevalence by age in the 2017 Ituri province morbidity study (n=586). Overall (green-solide line), female (red-dash line), and male (maroon-long-dash line).

The age distribution of reported diarrhoea and blood in stool, as well as the ultrasonographically assessed hepato- and splenomegaly displayed an age distribution resembling the *S. mansoni* infection with peaks in the adolescent and adult age groups (Figure 6.3).

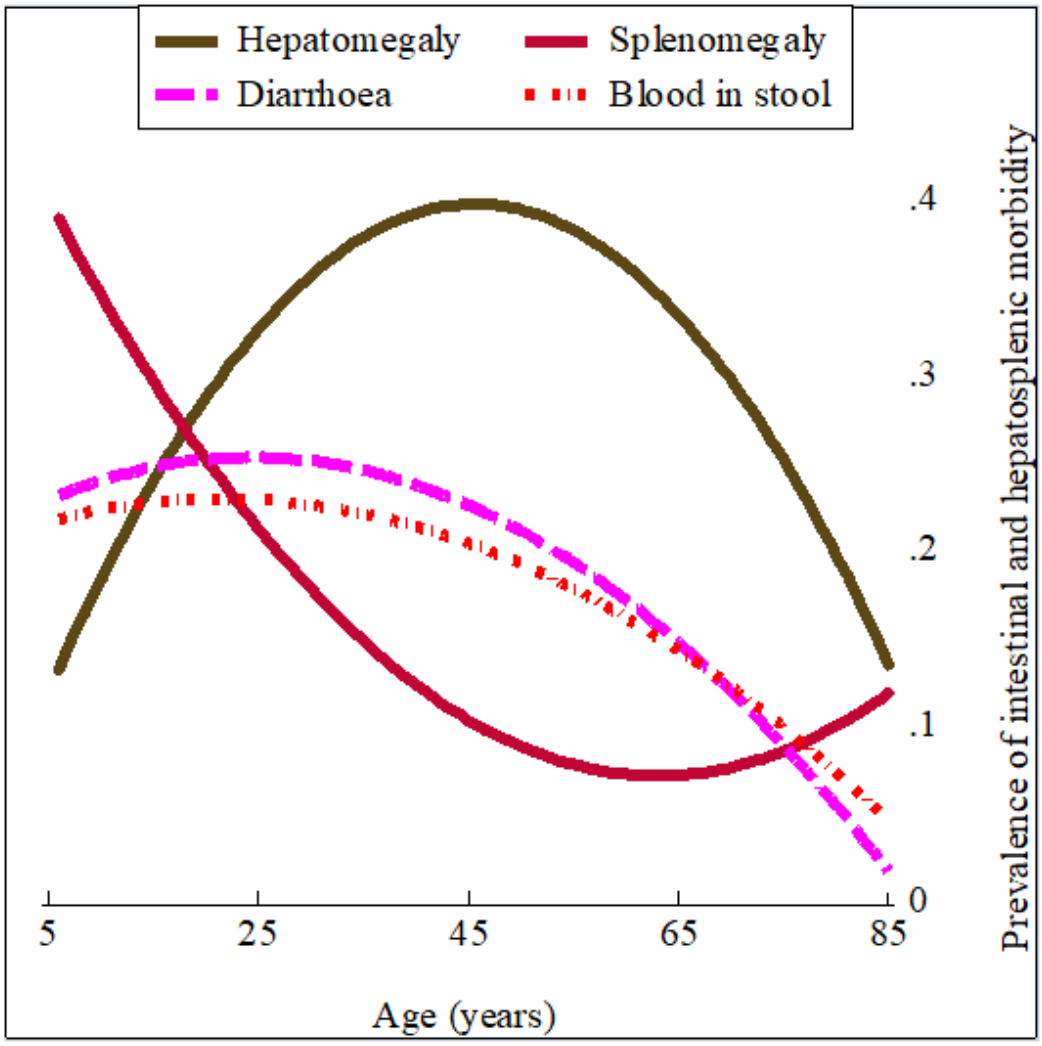

Figure 6.3: Age distribution of intestinal and hepatosplenic morbidity in the 2017 Ituri province morbidity study (n=586). Hepatomegaly (olive-solide line), splenomegaly (cranberry-solide line), diarrhoea (magenta-long-dash line), and blood in stool (red-short-dash-dot line).

The risk analysis considered the results of the combined diagnostic approach (Table 6.3). The results were consistent with those of the single diagnostic approaches, using Kato-Katz or POC-CCA. However, there were some differences. First, intestinal morbidity indicators, such as diarrhoea, were significantly associated with an *S. mansoni* infection (OR 1.69, 95% CI 1.03–2.78, *P*=0.038). This was also the case when the results of the Kato-Katz diagnostic approach were considered (OR 1.85, 95% CI 1.22–2.79, *P*=0.003). However, the association was not significant (OR 1.42, 95% CI 0.93–2.16, *P*=0.101) when using the results of the POC-CCA diagnostic approach. Other indicators, including the presence of blood in stool, abdominal pain, and history

of hematemesis, showed no association, regardless of the diagnostic approach used. Second, hepatomegaly was not significantly associated with an *S. mansoni* infection when using the combined diagnostic approach (OR 1.38, 95% CI 0.87–2.17, *P*=0.168), the POC-CCA diagnostic approach (OR 1.33, 95% CI 0.89–1.98, *P*=0.158), or the Kato-Katz diagnostic approach (OR 1.41, 95% CI 0.96–2.06, *P*=0.079). Splenomegaly was significantly associated with an *S. mansoni* infection when using the Kato-Katz diagnostic approach (OR 1.61, 95% CI 1.09–2.39, *P*=0.017). The association was not statistically significant when using the combined (OR 1.27, 95% CI 0.81–2.01, *P*=0.302) or POC-CCA (OR 1.17, 95% CI 0.78–1.74, *P*=0.451) diagnostic approaches. Third, an abnormal liver parenchyma pathology (combined patterns E/F) was significantly associated with *S. mansoni* infection when using the Kato-Katz diagnostic approach (OR 2.25, 95% CI 1.05–4.80, *P*=0.032). The association was not significant when using POC-CCA (OR 1.20, 95% CI 0.58–2.47, *P*=0.618) or the combined approach (OR 1.25, 95% CI 0.55–2.84, *P*=0.593).

Ten variables were included in the multivariable logistic regression analysis, the results of which are displayed in Table 6.3. Age was negatively associated with *S. mansoni* infection (adjusted odds ratio [aOR] 0.98; 95% CI 0.96–0.99, *P*=<0.001), while gender was not significantly associated with *S. mansoni* infection (aOR 1.15; 95% CI 0.74–1.79, *P*=0.524).

Of the morbidity indicators investigated, diarrhoea (aOR 1.69; 95% CI 0.99–2.89, *P*=0.053) and hepatomegaly (aOR 1.58; 95% CI 0.96–2.61, *P*=0.071) were associated with *S. mansoni* infection with a borderline significance level. Patients with abnormal liver parenchyma patterns (aOR 1.13; 95% CI 0.98–1.31, *P*=0.100) did not have an increased risk for *S. mansoni* infection.

Table 6.3. Morbidity associated with *S. mansoni* infection in the 2017 study based on Kato-Katz and POC-CCA diagnostic approaches. Results of the multivariable analysis of data from 13 purposively selected villages in Ituri province (n=586).

Risk factors	aOR (95% CI)	Std. Err.	z	p-value
Demographic risk factors				
Age	0.98 (0.96–0.99)	0.006	-3.64	<0.001
Gender (Male/Female)	1.15 (0.74–1.79)	0.259	0.64	0.524
Anthropometric risk factors				
BMI	1.00 (0.95–1.06)	0.027	0.15	0.878
Clinical finding				
Diarrhoea	1.69 (0.99–2.89)	0.461	1.94	0.053
Blood in stool	0.90 (0.54–1.50)	0.237	-0.41	0.683
Ultrasound findings				
Hepatomegaly (Yes/No)	1.58 (0.96–2.61)	0.404	1.80	0.071
Splenomegaly (Yes/No)	0.91 (0.55–1.50)	0.230	-0.37	0.712
Ascites (Yes/No)	0.21 (0.03–1.69)	0.225	-1.46	0.144
Liver pathology (Yes/No)	1.13 (0.98–1.31)	0.085	1.65	0.100
Co-infection				
Hookworm (Yes/No)	0.08 (0.01–0.81)	0.093	-2.14	0.033

aOR: adjusted odds ratio in multivariable analysis; CI: confidence interval. BMI, body mass index (continuous variable).

At the village level, the prevelance of hepatomegaly (Figure 6.4) and splenomegaly (Figure 6.5) increased with the prevalence of *S. mansoni* infection. Four patients were diagnosed with ascites; all of whom were residents of villages where overall *S. mansoni* prevalence exceeded 80%.

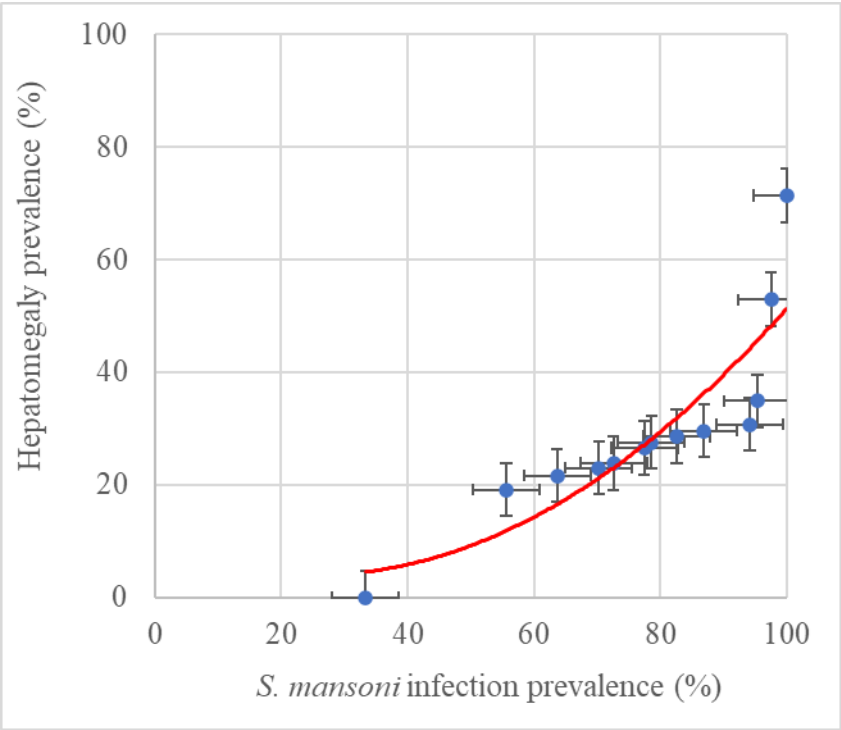

Figure 6.4: Association of hepatomegaly and *S. mansoni* infection prevalence at village level in the 2017 Ituri morbidity study (n=586).

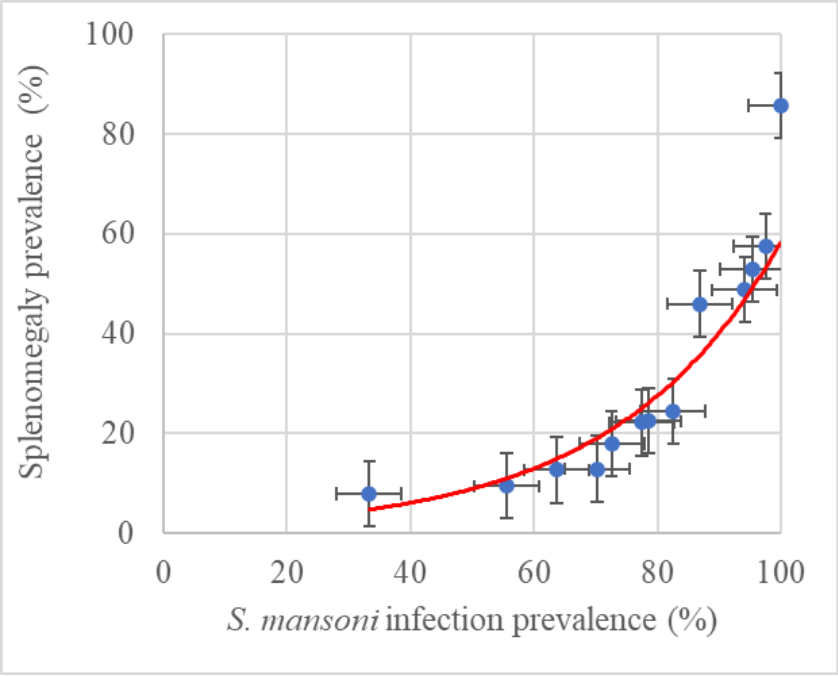

Figure 6.5: Association of splenomegaly and *S. mansoni* infection prevalence at village level in the 2017 Ituri morbidity study (n=586).

S. mansoni infection intensity varied greatly among individuals, with a maximum of 4,497.6 EPG and a mean infection intenstity of 109.7 EPG. Table 6.4 presents the infection intensity according to gender, age, helminth co-infection and morbidity categories. Infection intensity levels were similar in the two gender groups (P=0.198). The age distribution of the infection intensity levels followed the age-infection prevalence curve. Heavy-intensity infections were mostly found (12.9%) among adolescents aged 10–14 years ,while no one in the oldest age group (50 years and older) had a heavy-intensity infection (*P*=0006). Heavy-intensity infections were significantly higher among patients co-infected with *Ascaris lumbricoides* (13.0%, *P*=0.005) and underweight participants (10.1%, *P*=0.033).

There was a significantly higher prevalence of reported diarrhoea (40.5%, *P*=0.004) and blood in the stool (52.4%, *P*<0.001) among patients in the heavy-intensity infection group compared to the other infection intensity groups.

Among patients with heavy-intensity infections, the prevalence of splenomegaly (57.1%) was significantly higher than among other infection intensity groups (*P*<0.001), while the prevalence of hepatomegaly (38.1%) was not statistically different compared with the other infection intensity groups (*P*=0.073). When stratified by age, patients with an enlarged liver and/or spleen bore a higher infection intensity burden compared to those with a normal-sized liver and spleen in the same age group (Figure 6.6 and Table S6.6). In general, younger patients (children <18 years old) experienced more high-intensity infections compared to those in older age groups (adults ≥ 18 years old).

Table 6.4: *S. mansoni* infection intensity by morbidity in the 2017 study. Study conducted in 13 purposively selected villages in Ituri province (n=586). Only results of the Kato-Katz diagnostic approach have been considered in this analysis.

Characteristics	*S. mansoni* infection intensity									
	Negative		Light		Moderate		Heavy			
	n	%	n	%	n	%	n	%	χ^2	p-value
Overall	239	40.8	216	36.9	89	15.2	42	7.2		
Gender										
Females	152	44.4	117	34.2	50	14.6	23	6.7		
Males	87	35.7	99	40.6	39	16.0	19	7.8	4.66	0.198
Age categories (years)										
6 – 9	50	40.7	47	38.2	18	14.6	8	6.5		
10 – 14	49	35.0	49	35.0	24	17.1	18	12.9		
15 – 19	19	28.0	25	37.3	17	25.4	6	9.0		
20 – 29	27	35.0	32	41.6	11	14.3	7	9.1		
30 – 39	31	45.6	26	38.2	9	13.3	2	2.9		
40 – 49	27	51.9	18	34.6	6	11.6	1	1.9		
≥50	36	61.0	19	32.2	4	6.8	0	0.0	36.68	0.006
Soil-transmitted helminths										
T. trichiura (Y/N)	2	0.8	1	0.5	0	0.0	0	0.0	1.18	0.758
Ascaris (Y/N)	0	0.0	0	0.0	0	0.0	1	2.4	13.0	0.005
Hookworm (Y/N)	3	1.3	1	0.5	0	0.0	0	0.0	2.21	0.530
Anthropometry (BMI)										
Obese	33	56.9	20	34.5	4	6.9	1	1.7		
Overweight	13	54.2	9	37.5	2	8.3	0	0.0		
Normal weight	96	40.7	89	37.7	37	15.7	14	5.9		
Underweight	97	36.2	98	36.5	46	17.2	27	10.1	18.15	0.033
Clinical findings										
Diarrhoea (Y/N)	41	17.2	56	25.9	23	25.8	17	40.5	13.11	0.004
Blood in stool (Y/N)	42	17.6	32	14.8	30	33.7	22	52.4	39.49	<0.001
Abdom. pain (Y/N)	124	51.9	107	49.5	52	58.4	26	61.9	3.53	0.317
Hematemesis (Y/N)	3	1.3	1	0.5	1	1.1	0	0.0	1.28	0.733
Ultrasound findings										
Hepatomegaly (Y/N)	54	22.6	65	30.1	20	22.5	16	38.1	6.95	0.073
Splenomegaly (Y/N)	48	20.1	48	22.2	28	31.5	24	57.1	28.88	<0.001
Ascites (Y/N)	3	1.3	0	0.0	1	1.1	0	0.0	3.19	0.364
Pattern A/B (Y/N)	146	44.5	120	36.6	43	13.1	19	5.8		
Pattern C/D (Y/N)	80	37.6	79	37.1	37	17.4	17	8.0		
Pattern E/F (Y/N)	10	26.3	13	34.2	9	23.7	6	15.8		
Fatty liver (Y/N)	2	33.3	4	66.7	0	0.0	0	0.0		
Other (Y/N)	1	100	0	0.0	0	0.0	0	0.0	28.01	0.140

BMI, body mass index; Abdom.: abdominal. Pattern A: normal; Pattern B: "starry sky"; Pattern C: "rings and pipe-stems"; Pattern D "highly echogenic ruff around portal bifurcation"; Pattern E "highly echogenic patches"; Pattern F: "highly echogenic bands and streaks – bird's claw"; Fatty

liver and Other non-identified pathology indicate pathology different from periportal fibrosis. [55, 337].

S. mansoni infection intensity varied considerably among patients with different liver parenchyma pathologies (Tables S6.5 and S6.7). In general, more heavy-intensity infections were observed among patients with more severe liver morbidity patterns. That is, the number of individuals with heavy-intensity infections increased with the severity of the liver parenchyma pattern, from normal liver parenchyma patterns A and B (5.8%), to the most severe ("bird's claw") patterns E and F (15.8%). The association was not statistically significant (P=0.107). When stratified by age, a clear association emerged between increased number of high-intensity infections and increasingly abnormal liver pathologies (Figure 6.7). Liver parenchyma worsened (from A and B normal patterns, to C and D mild PPF patterns, and to E and F severe PPF patterns) as the median infection intensity increased. However, taken alone, patients with pattern F had similar- or lower-intensity infections than patients with less severe morbidity patterns (Table S6.7).

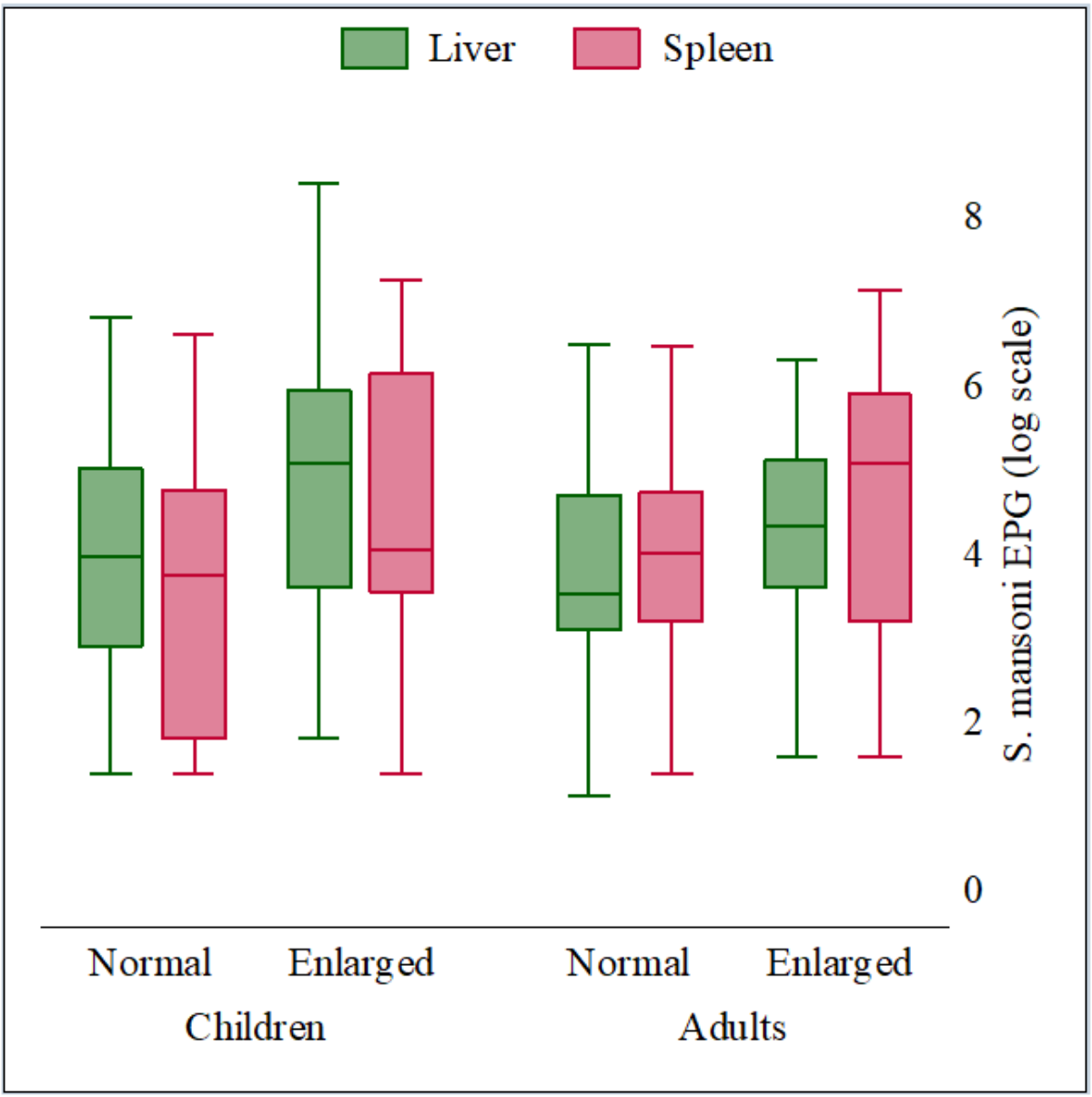

Figure 6.6: *S. mansoni* infection intensity by hepatomegaly and splenomegaly and age in the 2017 Ituri morbidity study (n=586). Hepatomegaly (green) and splenomegaly (cranberry).

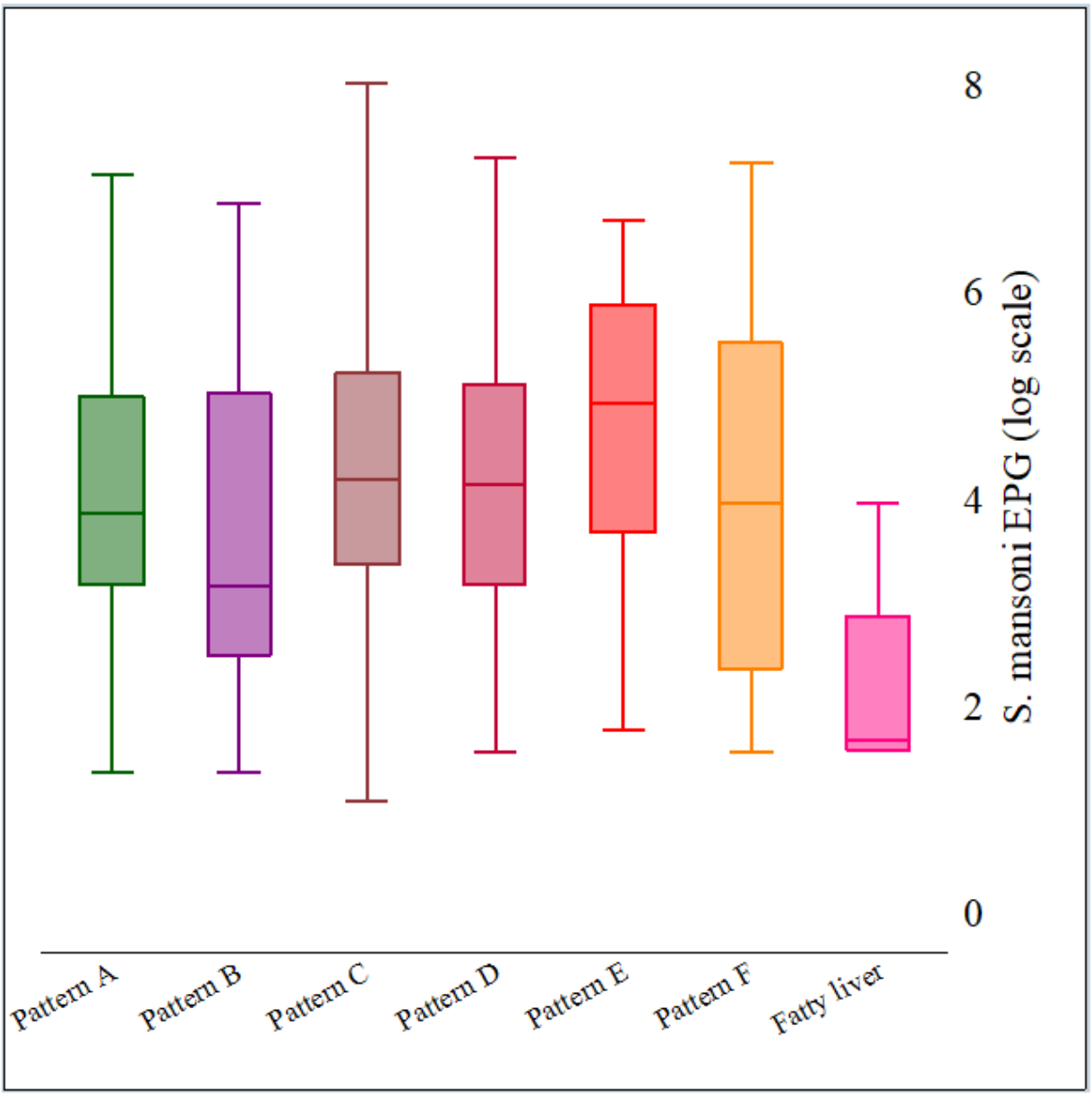

Figure 6.7: *S. mansoni* infection intensity by liver parenchyma patterns and age in the 2017 Ituri morbidity study (n=586). Patterns A (dark green) and B (purple) : normal; Patterns C (maroon) and D (cranberry): mild PPF; Patterns E (red) and F (orange): severe PPF; Fatty liver (pink) [337].

6.6 Discussion

In the World Health Organization's roadmap for neglected tropical diseases 2012-2030, the focus with regard to schistosomiasis is morbidity control. This is especially the case for the African region, where transmission levels in several countries, including the Democratic Republic of the Congo, are high, and elimination is not yet on the horizon. A key recommendation is to administer preventive chemotherapy, namely praziquantel. Mass treatment of a risk-exposed population at regular intervals prevents the development of high-intensity infections and hence, of morbidity [339, 340].

Our study provides, for the first time, comprehensive baseline data showing a high intestinal and hepatosplenic morbidity burden associated with *S. mansoni* infection in Ituri province, at both the individual and community levels.

Although minimizing morbidity is the target of schistosomiasis control efforts, control programmes rarely collect (baseline) and monitor morbidity data. Instead, they largely rely on monitoring infection intensities, which are linked to morbidity and much easier to assess than intestinal and hepatosplenic morbidity. Consequently, little is known about the morbidity burden of schistosomiasis [334].

Control programmes have been conducted successfully in colonial time, even with means that today may be considered outdated. However, with these means the disease was well controlled [118]. Since the independence of the country, no large-scale activity aimed at combating the disease has ever been undertaken until 2012. Since this date, a public control program targeting school children started in the country and it was launched in the Ituri province in 2016.

In this study, we assessed the magnitude of the intestinal and hepatosplenic morbidity burden in Ituri province, DRC, and investigated its associations with *S. mansoni* infection status. To that end, we conducted a cross-sectional study in 13 *S. mansoni* endemic villages. We enrolled all household member six years and older and assessed their *S. mansoni* infection status and their intestinal and hepatosplenic morbidity.

We found a high degree of intestinal and hepatosplenic morbidity. About one quarter of the study participants reported diarrhoea (23.4%) and blood in the stool (21.5%). Upon ultrasonography examination, almost one-quarter was diagnosed with hepatomegaly (26.5%); almost two-thirds (25.3%) had splenomegaly, and more than half (42.8%) had abnormal liver parenchyma (pattern C–F). Five patients reported an experience of hematemesis and four patients had ascites.

In our study population, we found a high prevalence of *S. mansoni* infection (76.6%). As the POC-CCA diagnostic approach does not provide information about infection intensity, the Kato-Katz diagnostic approach was used to analyze infection intensity. Light-, moderate- and heavy-intensity infections were diagnosed in high frequencies of 36.9%, 15.2% and 7.2%, respectively. Only a few cases of soil-transmitted helminths were diagnosed during this study and likely had a very small impact on the morbidity findings. *S. mansoni* infection prevalence and intensity was highest in the adolescent and young adult age groups. The prevalence of intestinal and hepatosplenic morbidity indicators showed a very similar age distribution (although hepato- and splenomegaly peaked in older age groups), and at village level, hepatosplenic morbidity prevalence increased with infection prevalence. Both observations suggesting a close link between morbidity and *S. mansoni* infection. Furthermore, at individual level, we found an increased risk of hepatomegaly and splenomegaly in *S. mansoni*-infected patients, confirming the association found at village level and the similarly shaped age distributions. The findings are also consistent with documented hepatosplenic morbidity associated with *S. mansoni* infection [341, 342]. Hence, providing further evidence that *S. mansoni* infection is a major contributor to the overall observed morbidity.

We found three notable differences in the risk results when relying on the diagnostic results from the Kato-Katz technique only. However, as we sought the respective advantages of a more specific (Kato-Katz) and a more sensitive (POC-CCA) diagnostic approach, we considered the results of the combined diagnostic approach. First, reported diarrhoea was significantly associated with *S. mansoni* infection; second, pathological changes of the liver parenchyma were associated with *S. mansoni* infection as was hepatomegaly; and third, splenomegaly was not associated with *S. mansoni* infection. Using the Kato-Katz test to diagnose *S. mansoni* infection reduces the overall sensitivity of the diagnostic approach due to the low sensitivity of the technique itself [343, 344]. Hence, on average, those diagnosed with an *S. mansoni* infection are more likely to have a higher infection intensity in comparison to the combined diagnostic approach. From these observations,

we see that subtle morbidity increases — such as reported diarrhoea and pathological changes in the liver parenchyma — become statistically significant. Indeed, for both morbidity indicators, we observed an association with *S. mansoni* infection intensity. Patients with diarrhoea had the highest prevalence of heavy-intensity infections (Table 2) and those with abnormal liver parenchyma patterns E–F had the highest mean infection intensities (Figure 4 and Table S7). However, the association between parasitological diagnosis and morbidity was much higher at the community (village) level than at the individual level.

In our study, we found that patients with abnormal liver parenchyma pattern F displayed lower *S. mansoni* infection intensity and risk compared to those with the E pattern. The highly echogenic bands and streaks corresponding to pattern F extend from the main portal vein and its bifurcation to the liver surface. Most commonly, this pattern manifests itself in very advanced cases of liver fibrosis and is frequently accompanied by other changes[55, 337]. Patients who displayed this pattern were often older, and thus had little contact with water, while some had already been treated multiple times with praziquantel. Consequently, they may have been free of infection or may have had light-intensity infections. It is also known that treatment with praziquantel can not halt the progression of organ damage in some individuals. This situation may be due to immunological and genetic factors, as well as other influences, including malaria, viral hepatitis and/or concomitant alcohol consumption [30, 345-347].

Quantifying schistosomiasis morbidity is a challenging [334, 348] and controversial matter [74]. Morbidity associated with *Schistosoma* infection is unspecific. Hence, the observed morbidity pattern might be provoked partially by or in combination with other pathogens, such as other helminth species, protozoa, bacteria, and viruses. Given that multiple infections are frequent in tropical Africa, a combination of infections is most likely responsible for the observed morbidity. In Ituri province, other parasitic infections, such as malaria (i.e. *Plasmodium falciparum*), and other infections with hepatosplenic affinity, such as viral hepatitis, are prevalent [30, 52, 59, 349] and may have contributed to the hepatosplenic morbidity pattern observed. The time gap between infection and the occurrence of measurable morbidity further complicates efforts to assess the association between infection and morbidity. Furthermore, organomegaly is sometimes described as normally present in children; it then regresses, and disappears in adulthood [30, 71, 350, 351].

Nevertheless, these findings do not in any way reduce the value of abdominal ultrasound in the diagnosis of liver pathologies associated with *S. mansoni* infection. However, ultrasonography devices are rarely available in poor-resource settings. In Ituri province, the device may be found only in the provincial hospital, and in some district hospitals and private clinics. During our field work, we found that the use of portable devices was feasible in the villages. However, in the most remote rural areas, usage is challenging due to the unavailability of electricity. Additional equipment, such as solar panels and rechargeable batteries, would be required. Despite these shortcomings, abdominal ultrasound yields crucial information about liver morbidity in the community and hence, indispensable information about the public health burden of *S. mansoni* infection [73]. Furthermore, severe cases would likely be diagnosed earlier and adequately addressed.

In our study, we encountered patients with severe complications from *S. mansoni* infection, which further underscore the importance of the infection's morbidity burden. Four people (0.9%) reported a history of hematemesis and two people (0.5%) reported ascites. The finding appears to corroborate the health service's statistics report from the Angumu health district (on the shore of Lake Albert), which declares that hematemesis is a frequent medical emergency and that adults have died after vomiting blood in this area. Oesophageal varices remain silent until they rupture and irreversible damage occurs [349]. Angumu health district is a remote area and well known for its high blood transfusion rates. Patients vomiting blood often reach the hospital too late, leading to the worst medical outcome.

The morbidity levels we observed are consistent with those measured by Ongom and Bradley [336], who found serious morbidity, including diarrhoea and abdominal pain, in a schistosomiasis endemic community on Lake Albert in Uganda. Other studies of *S. mansoni* endemic communities outside of the DRC present similar morbidity levels [71-73]. Very few studies of morbidity due to schistosome infection in DRC exist [349, 352]. Our study contributes to the country's knowledge base and may offer a baseline for future intervention studies to determine the exact extent of morbidity associated with *S. mansoni* infection.

Our study presents some limitations. We conducted our study in purposively sampled villages known to have a high prevalence of *S. mansoni*. Thus, the examined population is not representative of the entire province but rather of high transmission areas. Furthermore, ongoing civil unrest in

Ituri province creates a challenging security situation, which only afforded us a short time in each village. For this reason, only one stool sample could be collected from each study participant. Finally, limited available resources did not allow us to examine participants for parasitic, bacterial, and viral co-infections, which could have helped to better explain the degree of morbidity linked to *S. mansoni* infection.

Other limitations include a lack of blood coinfection diagnosis and a body mass index (BMI) cut-off that did not take into account variations among the study population, as recommended [353]. Concerning the first, we needed to minimize invasive procedures and exclude vulnerable individuals. Indeed, blood sampling is a very sensitive topic in our study area, as the population is reluctant to have blood drawn. Blood sampling would have required a lot of time and effort and would have greatly reduced the level of compliance among the study participants. Hence, we did not diagnose coinfections such as malaria [354], viral hepatitis [355], human immune virus (HIV), and other similar diseases endemic in the province. Despite these possible confounding factors, the association of *S. mansoni* infection with hepatosplenic and intestinal morbidity remains highly significant.

The BMI cut-off values were randomly defined by four categories, including underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30 kg/m²). This random clustering may have introduced a selection bias in our results. However, in the risk analysis, we used the BMI as a continuous variable rather than a categorical one.

Conclusion

Schistosomiasis mansoni is an important public health problem in Ituri province, yet appropriate control measures have not yet been fully implemented. A public programme for controlling the disease was launched in the province in 2016. It is based on preventive chemotherapy and aims to control infection among school children. Our results show that both infection rates and related morbidity are very high in the province. The situation calls for vigorous and efficient control measures to address this scourge.

Acknowledgements

We are grateful to all the study participants both in 2016 and in 2017. Our sincere thanks to the research teams in this two-years period. We thank the Provincial Health Division officers, all the Health Districts officers, and the Institut Supérieur des Techniques Médicales de Nyankunde staff for their support and the local authorities for their support during the fieldwork.

Funding

The study was funded by private funds (Poverty Fund, Switzerland). The funders had no role in study design, data collection and data analysis, decision to publish, or preparation of the manuscript.

Competing interest

No authors have competing interests.

Supplementary information: Tables

Table S6.1: Univariable associations with *S. mansoni* infection in the 2017 morbidity study based on Kato-Katz test only. Study conducted in 13 purposively selected villages in Ituri province (n=586). Only results of Kato-Katz (KK) diagnostic approach have been considered.

Characteristics	*S. mansoni* (+) N=347		*S. mansoni* (-) N=239			
	n	%	n	%	OR (95% CI)	p-value
Gender*						
Females	190	55.6	152	44.4	1.0	
Males	157	64.3	87	35.7	1.44 (1.03–2.03)	0.033
Age categories (years)*						
6 – 9	73	59.4	50	40.6	1.0	
10 – 14	91	65.0	49	35.0	1.27 (0.77–2.10)	0.346
15 – 19	48	71.6	19	28.4	1.73 (0.91–3.30)	0.093
20 – 29	50	64.9	27	35.1	1.27 (0.70–2.29)	0.431
30 – 39	37	54.4	31	45.6	0.81 (0.45–1.49)	0.510
40 – 49	25	48.1	27	51.9	0.63 (0.33–1.22)	0.171
≥50	23	39.0	36	61.0	0.44 (0.23–0.84)	0.010
STH						
T. trichiura (Y/N)	1	0.3	2	0.84	0.34 (0.03–3.81)	0.361
A. lumbricoides (Y/N)	1	0.3	0	0.0	na	
Hookworm (Y/N) *	4	0.3	3	1.3	0.23 (0.02–2.21)	0.163
Anthropometry (BMI)*						
Obese (Y/N)	25	7.2	33	13.8	1.0	
Overweight (Y/N)	11	3.2	13	5.4	1.12 (0.42–3.92)	0.822
Normal weight (Y/N)	140	40.4	96	40.2	1.92 (1.07–3.46)	0.026
Underweight (Y/N)	171	49.3	97	40.6	2.33 (1.30–4.18)	0.004
Clinical findings						
Diarrhoea (Y/N) *	96	27.7	41	17.2	1.85 (1.22–2.79)	0.003
Blood in stool (Y/N) *	84	24.2	42	17.6	1.50 (0.99–2.27)	0.055
Abdominal pain (Y/N)	185	53.3	124	51.9	1.06 (0.76–1.47)	0.733
Hematemesis (Y/N)	2	0.6	3	1.3	0.46 (0.08–2.76)	0.380
Ultrasound findings						
Hepatomegaly (Y/N) *	101	29.1	54	22.6	1.41 (0.96–2.06)	0.079
Splenomegaly (Y/N) *	100	28.8	48	20.1	1.61 (1.09–2.39)	0.017
Ascites (Y/N) *	1	0.3	3	1.3	0.23 (0.02–2.21)	0.163
Pattern A/B (Y/N) *	182	52.5	146	61.1	1.0	
Pattern C/D (Y/N)	133	38.3	80	33.5	1.33 (0.94–1.95)	0.109
Pattern E/F (Y/N)	28	8.1	10	4.2	2.25 (1.05–4.80)	0.032
Fatty liver (Y/N)	2	0.8	4	1.2	1.38 (0.25–7.62)	0.709
Other (Y/N)	1	0.4	0	0.0	na	

* Included in the multivariable analysis. BMI, body mass index; na, not applicable; Pattern A: normal; Pattern B: “starry sky”; Pattern C: “rings and pipe-stems”; Pattern D “highly echogenic ruff around portal bifurcation”; Pattern E “highly echogenic patches”; Pattern F: “highly

echogenic bands and streaks – bird's claw"; Fatty liver (pattern Y) and other abnormality (pattern Z) indicate pathology different from periportal fibrosis [55, 337].

Table S6.2. Risk factors for morbidity due to *Schistosoma mansoni* infection, 2017 study. Results of the multivariable analysis of risk factors for morbidity due to *Schistosoma mansoni* infection among participants from 13 villages in Ituri province (n=586). Only results of Kato-Katz (KK) diagnostic approach have been considered.

Risk factors	aOR (95% CI)	Std. Err.	z	p-value
Demographic risk factors				
Age	0.98 (0.97–0.99)	0.006	-3.48	<0.001
Gender (Male/Female)	1.43 (1.00–2.12)	0.279	1.94	0.052
Anthropometric risk factors				
BMI	1.00 (0.95–1.04)	0.024	-0.18	0.857
Clinical finding				
Diarrhoea	1.76 (1.12–2.74)	0.400	2.47	0.013
Blood in stool	1.13 (0.26–1.77)	0.261	0.52	0.606
Ultrasound findings				
Hepatomegaly (Yes/No)	1.52 (0.99–2.32)	0.329	1.93	0.053
Splenomegaly (Yes/No)	1.12 (0.73–1.72)	0.246	0.50	0.619
Ascites (Yes/No)	0.13 (0.01–1.37)	0.156	-1.70	0.089
Liver pathology (Yes/No)	1.20 (1.06–1.37)	0.080	2.80	0.005
Co-infection				
Hookworm (Yes/No)	0.20 (0.02–2.06)	0.240	-1.35	0.177

aOR: adjusted odds ratio; CI: confidence interval. BMI, body mass index (only taken as continious variable).

Table S6.3: Morbidity association with *S. mansoni* infection in the 2017 study based on POC-CCA diagnostic approach. Results of the univariable analysis of data from 13 purposively selected villages of Ituri province (n=586).

Characteristics	*S. mansoni* (+) N=385		*S. mansoni* (-) N=201			
	n	%	n	%	OR (95% CI)	p-value
Gender*						
Females	222	57.7	120	59.7	1.0	
Males	163	42.3	81	40.3	1.09 (0.77-1.54)	0.635
Age categories (years)*						
6 – 9	80	20.8	43	21.4	1.0	
10 – 14	105	27.3	35	17.4	1.61 (0.94-2.76)	0.078
15 – 19	45	11.7	22	11.0	1.10 (0.58-2.07)	0.769
20 – 29	57	14.8	20	10.0	1.53 (0.81-2.89)	0.184
30 – 39	40	10.4	28	14.0	0.77 (0.42-1.42)	0.396
40 – 49	30	7.8	22	11.0	0.73 (0.38-1.43)	0.359
≥50	28	7.3	31	15.4	0.49 (0.26-0.92)	0.024
STH						
T. trichiura (Y/N)	1	0.3	2	1.0	0.26 (0.02-2.89)	0.237
A. lumbricoides (Y/N)	1	0.3	0	0.0	na	
Hookworm (Y/N) *	1	0.3	3	1.5	0.17 (0.02-1.68)	0.086
Anthropometry (BMI)*						
Obese (Y/N)	29	7.5	29	14.4	1.0	
Overweight (Y/N)	17	4.4	7	3.5	2.43 (0.85-6.91)	0.086
Normal weight (Y/N)	155	40.3	81	40.3	1.91 (1.06-3.44)	0.027
Underweight (Y/N)	184	47.8	84	41.8	2.19 (1.22-3.93)	0.007
Clinical findings						
Diarrhoea (Y/N) *	98	25.5	39	19.4	1.42 (0.93-2.16)	0.101
Blood in stool (Y/N)	91	23.6	35	17.4	1.47 (0.95-2.27)	0.082
Abdominal pain (Y/N)	203	52.7	106	52.7	1.00 (0.71-1.41)	0.998
Hematemesis (Y/N)	4	1.0	1	0.5	2.10 (0.23-18.96)	0.499
Ultrasound findings						
Hepatomegaly (Y/N) *	109	28.3	46	22.9	1.33 (0.89-1.98)	0.158
Splenomegaly (Y/N) *	101	26.2	47	23.4	1.17 (0.78-1.74)	0.451
Ascites (Y/N) *	2	0.5	2	1.0	0.52 (0.07-3.73)	0.507
Pattern A/B (Y/N) *	211	54.8	117	58.2	1.0	
Pattern C/D (Y/N)	145	37.7	68	33.8	1.18 (0.82–1.71)	0.370
Pattern E/F (Y/N)	26	6.8	12	6.0	1.20 (0.58–2.47)	0.618
Fatty liver (Y/N)	4	2.0	2	0.5	0.26 (0.05–1.42)	0.094
Other (Y/N)	0	0.0	1	0.3	na	

* Included in the multivariable analysis. BMI, body mass index; na, not applicable; Pattern A: normal; Pattern B: "starry sky"; Pattern C: "rings and pipe-stems"; Pattern D "highly echogenic ruff around portal bifurcation"; Pattern E "highly echogenic patches"; Pattern F: "highly echogenic bands and streaks – bird's claw"; Fatty liver (pattern Y) and other abnormality (pattern Z) indicate pathology different from periportal fibrosis [55, 337].

Table S6.4. Risk factors for morbidity due to *Schistosoma mansoni* infection, 2017 study. Results of the multivariable analysis of risk factors for morbidity due to *Schistosoma mansoni* infection among participants from 13 villages in Ituri province (n=586). Results of POC-CCA diagnostic approach have been considered.

Risk factors	aOR (95% CI)	Std. Err.	z	p-value
Demographic risk factors				
Age	0.98 (0.97–0.99)	0.006	-3.69	<0.001
Gender (Male/Female)	1.07 (0.73–1.57)	0.208	0.35	0.727
Anthropometric risk factors				
BMI	1.01 (0.96–1.07)	0.025	0.30	0.762
Clinical finding				
Diarrhoea	1.29 (0.83–2.03)	0.296	1.13	0.260
Blood in stool	1.31 (0.83–2.10)	0.313	1.15	0.250
Ultrasound findings				
Hepatomegaly (Yes/No)	1.55 (1.01–2.40)	0.345	1.99	0.046
Splenomegaly (Yes/No)	0.84 (0.54–1.29)	0.185	-0.80	0.423
Ascites (Yes/No)	0.56 (0.07–4.26)	0.579	-0.56	0.573
Liver pathology (Yes/No)	1.04 (0.91–1.18)	0.068	0.54	0.589
Co-infection				
Hookworm (Yes/No)	0.15 (0.01–1.50)	0.174	-1.62	0.106

aOR: adjusted odds ratio; CI: confidence interval. BMI, body mass index (only taken as continious variable).

Table S6.5: Prevalence of periportal fibrosis (PPF) by age, sex, village, and *S. mansoni* infection status in the 2017 study. Results from 13 purposively selected villages of Ituri province (n=586). Prevalence with the combined diagnostic approach and intensity with Kato-Katz diagnostic approach.

Characteristics	Periportal fibrosis (PPF)			*S. mansoni* infection status					
				Prevalence			Intensity*		
	Overall (%)	Female (%)	Male (%)	Overall (%)	Female (%)	Male (%)	Overall	Female	Male
Overall	251 (42.8)	147 (43.0)	104 (42.6)	449 (76.6)	257 (75.2)	192 (78.7)	109.7	91.1	135.8
Age categories									
6 – 9	46 (37.4)	26 (41.3)	20 (33.3)	92 (74.8)	45 (71.4)	47 (78.3)	112.6	57.0	170.9
10 – 14	52 (37.1)	29 (40.9)	23 (33.3)	119 (85.0)	60 (84.5)	59 (85.5)	156.1	131.1	181.8
15 – 19	34 (50.8)	17 (44.7)	17 (58.6)	54 (80.6)	28 (73.7)	26 (89.7)	133.9	140.8	124.9
20 – 29	40 (52.0)	33 (52.4)	7 (50.0)	63 (81.8)	50 (79.4)	13 (92.9)	110.6	111.9	104.9
30 – 39	29 (42.7)	17 (37.8)	12 (52.2)	48 (70.6)	31 (68.9)	17 (73.9)	76.3	94.7	40.4
40 – 49	21 (40.4)	12 (37.5)	9 (45.0)	35 (67.3)	22 (68.8)	13 (65.0)	85.2	18.8	191.4
≥50	29 (49.2)	13 (43.3)	16 (55.2)	38 (64.4)	21 (70.0)	17 (58.6)	25.1	32.7	17.1
Villages									
Bankoko	8 (29.6)	3 (20.0)	5 (41.7)	9 (33.3)	3 (20.0)	6 (50.0)	3.2	0.3	6.8
Lumumba	5 (22.7)	3 (20.0)	2 (28.6)	14 (63.4)	11 (73.3)	3 (42.9)	60.0	57.7	64.9
Simbilyabo	14 (22.2)	6 (15.4)	8 (33.3)	35 (55.6)	23 (59.0)	12 (50.0)	46.1	48.3	42.6
Mangenengene	20 (54.1)	12 (54.6)	8 (53.3)	26 (70.3)	16 (72.7)	10 (66.7)	14.6	10.6	20.5
Kadjugi	23 (37.1)	14 (35.9)	9 (39.1)	45 (72.6)	26 (66.7)	19 (82.6)	94.3	29.7	203.8
Kindia	8 (12.7)	7 (21.2)	1 (3.3)	52 (82.5)	26 (78.8)	26 (86.7)	30.7	38.0	22.7
Gupe	65 (63.7)	33 (57.9)	32 (71.1)	79 (77.5)	44 (77.2)	35 (77.8)	97.3	124.2	63.3
Sukisa	11 (26.2)	10 (38.5)	1 (6.3)	33 (78.6)	21 (80.8)	12 (75.0)	29.4	20.2	44.4
Ngezi	33 (54.1)	23 (65.7)	10 (38.5)	53 (86.9)	28 (80.0)	25 (96.2)	102.5	65.9	151.9
Mambau	8 (47.1)	6 (46.2)	2 (50.0)	16 (94.1)	13 (100)	3 (75.0)	193.4	249.6	11.1
Mandima	25 (62.5)	17 (60.7)	8 (66.7)	39 (97.5)	27 (96.4)	12 (100)	150.9	156.6	137.6
Pekele	26 (60.5)	11 (64.7)	15 (57.7)	41 (95.4)	16 (94.1)	25 (96.2)	519.3	459.1	558.7
Ndaru-Muswa	5 (71.4)	2 (66.7)	3 (75.0)	7 (100)	3 (100)	4 (100)	370.3	136.0	546.0

*Note: Intensity = arithmetic mean egg peg gram (EPG) count.

Table S6.6: Prevalence of hepatomegaly, splenomegaly and overall organomegaly by age, sex, village, and *S. mansoni* infection status in the 2017 study. Results from 13 purposively selected villages of Ituri province (n=586). Prevalence with the combined diagnostic approach.

Characteristics		No	Organ enlargement status						*S. mansoni* infection status	
			*Organomegaly		Hepatomegaly		Splenomegaly	*Hepatosplenomegaly	Prevalence	Intensity
			n	%	n	%	n %	n %	n %	mean EPG
Overall		586	254	(43.3)	155	(26.5)	148 (25.3)	49 (8.4)	449 (76.6)	109.7
Sex	Female	342	131	(38.3)	75	(21.9)	85 (24.9)	29 (8.5)	257 (75.2)	91.1
	Male	244	123	(50.4)	80	(32.8)	63 (25.8)	20 (8.2)	192 (78.7)	135.8
Age	6 – 9	123	60	(48.8)	23	(18.7)	50 (40.7)	13 (10.6)	47 (78.3)	112.6
	10 – 14	140	44	(31.4)	13	(9.3)	40 (28.6)	9 (6.4)	59 (85.5)	156.1
	15 – 19	67	40	(59.7)	31	(46.3)	20 (29.9)	11 (16.4)	26 (89.7)	133.9
	20 – 29	77	35	(45.5)	24	(31.2)	17 (22.1)	6 (7.8)	13 (92.9)	110.6
	30 – 39	68	30	(44.1)	27	(39.7)	10 (14.7)	7 (10.3)	17 (73.9)	76.3
	40 – 49	52	22	(42.3)	17	(32.7)	7 (13.5)	2 (3.9)	13 (65.0)	85.2
	≥50	59	23	(39.0)	20	(33.9)	4 (6.8)	1 (1.7)	17 (58.6)	25.1
Village	Bankoko	27	10	(37.0)	8	(29.6)	6 (22.2)	4 (14.8)	6 (50.0)	3.2
	Lumumba	22	5	(22.7)	0	(0.0)	5 (22.7)	0 (0.0)	3 (42.9)	60.0
	Simbilyabo	63	19	(30.2)	12	(19.1)	8 (12.7)	1 (1.6)	12 (50.0)	46.1
	Mangenengene	37	22	(59.5)	8	(21.6)	17 (46.0)	3 (8.1)	10 (66.7)	14.6
	Kadjugi	62	24	(38.7)	19	(30.7)	8 (12.9)	3 (4.8)	19 (82.6)	94.3
	Kindia	63	17	(27.0)	15	(23.8)	5 (7.9)	3 (4.8)	26 (86.7)	30.7
	Gupe	102	47	(46.1)	27	(26.5)	25 (24.5)	5 (4.9)	35 (77.8)	97.3
	Sukisa	42	14	(33.3)	12	(28.6)	4 (9.5)	2 (4.8)	12 (75.0)	29.4
	Ngezi	61	22	(36.1)	14	(23.0)	11 (18.0)	3 (4.9)	25 (96.2)	102.5
	Mambau	17	13	(76.5)	9	(52.9)	9 (52.9)	5 (29.4)	3 (75.0)	193.4
	Mandima	40	27	(67.5)	11	(27.5)	23 (57.5)	7 (17.5)	12 (100)	150.9
	Pekele	43	28	(65.1)	15	(34.9)	21 (48.8)	8 (18.6)	25 (96.2)	519.3
	Ndaru-Muswa	7	6	(85.7)	5	(71.4)	6 (85.7)	5 (71.4)	4 (100)	370.3

* Organomegaly = either hepatomegaly or splenomegaly; hepatosplenomegaly = simultaneous hepatomegaly and splenomegaly.

Table S6.7: Liver pattern prevalence and association with *S. mansoni* infection intensity in the 2017 study. Study conducted in 13 purposively selected villages in Ituri province (n=586). Results of the Kato-Katz diagnostic approach have been considered in this analysis.

Characteristics	Number (%)	*S. mansoni* infection intensity									
		Negative		Light		Moderate		Heavy			
		n	%	n	%	n	%	n	%	χ^2	p-value
Overall	586 (100)	239	40.8	216	36.9	89	15.2	42	7.2		
Liver patterns											
Pattern A	267 (45.6)	124	46.4	92	34.5	36	13.5	15	5.6		
Pattern B	61 (10.4)	22	36.1	28	45.9	7	11.5	4	6.6		
Pattern C	156 (26.6)	61	39.1	56	35.9	26	16.7	13	8.3		
Pattern D	57 (9.7)	19	33.3	23	40.4	11	19.3	4	7.2		
Pattern E	23 (3.9)	3	13.4	8	34.8	8	34.8	4	17.4		
Pattern F	15 (2.6)	7	46.7	5	33.3	1	6.7	2	13.3		
Fatty liver	6 (1.0)	2	33.3	4	66.7	0	0.0	0	0.0		
Other	1 (0.4)	1	100	0	0.0	0	0.0	0	0.0	28.01	0.140

Pattern A: normal; Pattern B: “starry sky”; Pattern C: “rings and pipe-stems”; Pattern D “highly echogenic ruff around portal bifurcation”; Pattern E “highly echogenic patches”; Pattern F: “highly echogenic bands and streaks – bird’s claw”; Fatty liver (pattern Y) and other abnormality (pattern Z) indicate pathology different from periportal fibrosis [55, 337].

Table S6.8: Univariable analysis of risk of liver patterns and periportal fibrosis (PPF) by *S. mansoni* infection status with different diagnostic approaches in the 2017 study. Results from 13 purposively selected villages of Ituri province (n=586). Analysis with Kato-Katz diagnostic approach, POC-CCA diagnostic approach, and with the combined diagnostic approach.

Liver patterns/ PPF	Kato-Katz OR (95% CI)	p-value	POC-CCA OR (95% CI)	p-value	Kato-Katz+POC-CCA OR (95% CI)	p-value
Pattern A	0.65 (0.47-0.91)	0.011	0.99 (0.70-1.39)	0.942	0.77 (0.53-1.14)	0.198
Pattern B	1.25 (0.72-2.17)	0.429	0.73 (0.42-1.25)	0.246	1.14 (0.60-2.18)	0.687
Pattern C	1.10 (0.76-1.60)	0.618	1.02 (0.69-1.50)	0.920	1.08 (0.69-1.66)	0.746
Pattern D	1.42 (0.80-2.54)	0.229	1.52 (0.82-2.82)	0.182	1.31 (0.66-2.60)	0.444
Pattern E	4.81 (1.40-16.53)	0.006	2.56 (0.85-7.65)	0.082	3.31 (0.76-14.38)	0.090
Pattern F	0.78 (0.28-2.19)	0.639	0.45 (0.16-1.25)	0.116	0.45 (0.16-1.28)	0.124
Fatty liver	1.73 (0.31-9.67)	0.525	0.26 (0.05-1.48)	0.103	1.74 (0.20-15.25)	0.611
Other	.	0.285		0.470		0.556
PPF categories						
Normal (A/B)	0.70 (0.50-0.98)	0.039	0.87 (0.62-1.23)	0.431	0.81 (0.55-1.20)	0.296
Mild (C/D)	1.23 (0.87-1.74)	0.230	1.18 (0.83-1.69)	0.360	1.17 (0.78-1.75)	0.441
Severe (E/F)	2.01 (0.95-4.23)	0.061	1.14 (0.56-2.31)	0.715	1.15 (0.52-2.58)	0.726
Overall PPF						
PPF (Yes/No)	1.43 (1.02-2.01)	0.036	1.21 (0.85-1.71)	0.284	1.20 (0.81-1.78)	0.356

Pattern A: normal; Pattern B: "starry sky"; Pattern C: "rings and pipe-stems"; Pattern D "highly echogenic ruff around portal bifurcation"; Pattern E "highly echogenic patches"; Pattern F: "highly echogenic bands and streaks – bird's claw"; Fatty liver (pattern Y) and other abnormality (pattern Z) indicate pathology different from periportal fibrosis [55, 337].

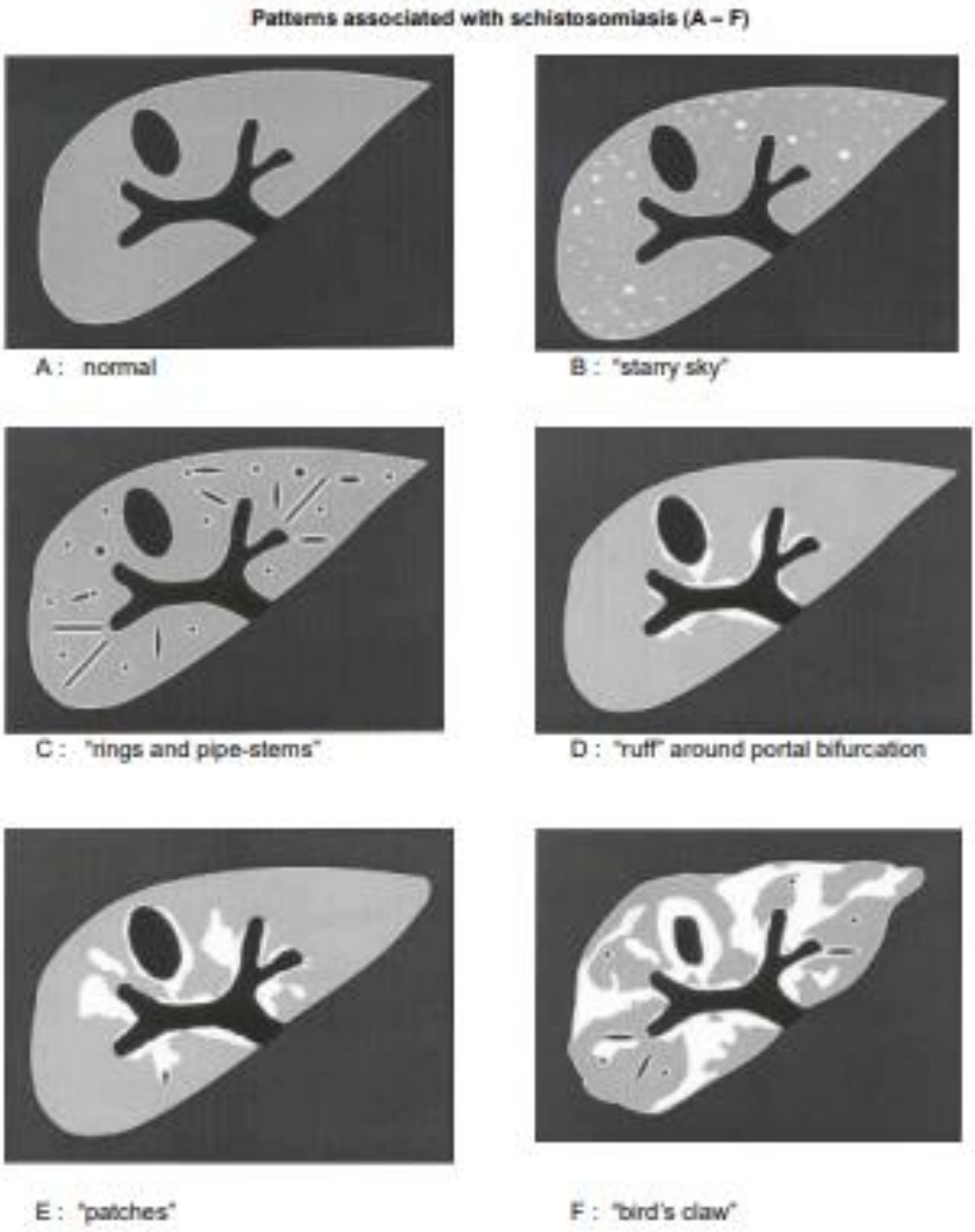

Figure S6.1: Liver image patterns associated with schistosomiasis, by [55].

7 Patients with severe schistosomiasis mansoni in the Ituri province, Democratic Republic of the Congo

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This article is in revision in the *BMC Infectious Diseases of Poverty journal*

7.1 Abstract

Background

Only rarely do reports document morbidity and severe complications arising in patients with chronic *Schistosoma mansoni* infection. We report on eight patients with severe morbidity associated with *S. mansoni* infection in Ituri province in northeastern Democratic Republic of Congo (DRC).

Methods

The patients were identified during a community-based survey in 2017; one patient was seen at the district hospital. After taking the patients' history, a clinical examination and an abdominal ultrasonographical examination were performed. *S. mansoni* infection was diagnosed using the Kato-Katz technique on stool and a point-of-case circulating cathodic *S. mansoni* antigen rapid urine test.

Results

The patients' ages ranged from 19 years to 57 years (mean of 36 years); three patients were men. Three patients reported an experience of hematemesis. Two patients were severely anaemic. All patients reported non-specific abdominal symptoms, such as diarrhoea (five patients), abdominal pain and blood in the stool (four patients each), and weight loss (five patients). Abdominal ultrasonography revealed ascites in four patients. All patients had portal hypertension with hepatosplenomegaly (six patients) or splenomegaly (two patients). Of the six patients for whom a liver parenchyma pattern could be assessed, five displayed pattern D–E and one patient displayed pattern B–C. The remaining two patients had severe ascites with a barely visible liver. An *S. mansoni* infection was confirmed in six patients. The infection ranged from light (24 eggs per gram of stool [EPG]) to heavy intensity (2,832 EPG). All patients were treated with praziquantel (40 mg/kg BW) and referred to the district hospital for follow-up. One patient with severe ascites died two weeks after we saw her. Six patients were lost to follow-up.

Conclusion

Our observations of patients with severe schistosomiasis document the urgent need for schistosomiasis control programmes that target vulnerable population groups.

Key words

Intestinal schistosomiasis; severe cases; hepatomegaly; splenomegaly; ascites; hematemesis; morbidity; mortality.

7.2 Introduction

Schistosomiasis is a widespread, neglected and debilitating disease [356]. *Schistosoma* infections afflict an estimated 220 million people worldwide, 90% of whom live in sub-Saharan Africa [357]. The morbidity and mortality associated with *Schistosoma* infection represents a major public health problem.

In intestinal schistosomiasis, i.e. *S. mansoni* in sub-Saharan Africa, the intestinal tract, liver and spleen are affected in 10–60% of chronically infected patients [358]. The symptoms of chronic infection may include abdominal pain, diarrhoea, bloody stools, anaemia, weight loss and stunting. Hepatomegaly and splenomegaly indicate a severe hepatosplenic pathology, including portal hypertension.

Severe complications associated with *S. mansoni* may include upper digestive haemorrhage with rapid blood loss, which can result in sudden death. A case series of 124 patients from Tanzania showed that during the chronic and terminal phase of intestinal schistosomiasis, patients with hepatosplenic involvement had a higher risk of dying from the disease [359]. The major cause of hematemesis is the rupture of oesophageal varices associated with portal hypertension [360]. Whereas, ascites, an accumulation of circulatory fluids in the peritoneal cavity, results from anatomic and pathophysiologic abnormalities [361].

Although *Schistosoma* infection is widespread in the Democratic Republic of Congo (DRC), relatively little recent information is available infection and disease burden, particularly regarding the rural parts of the country. However, a large cross-sectional study was recently performed across Ituri province in northeastern DRC. The prevalence of S. mansoni infection was higher in the South and East than in the North and West of the province, reaching rates of more than 90% in villages along the shore of Lake Albert [362]. In an in-depth follow-up study, high intestinal and hepatosplenic morbidity burdens were observed [363]. Overall, about one quarter of the study population reported diarrhoea and blood in the stool. Abdominal ultrasound examinations revealed that one quarter had hepatomegaly and that more than half of the study population had splenomegaly and abnormal liver parenchyma.

Here, we report on eight patients with severe complications due to *S. mansoni* infection, residing in villages with high *S. mansoni* prevalence in the eastern and southern regions of Ituri province, DRC.

7.3 Methods

Ethical considerations

This study was approved by the Swiss Ethical Commission (Ref. No. UBE–15/78) and by the University of Kisangani's Research Ethical Commission (Ref No: CER/003/GEAK/2016). Research authorization was granted by the Nyankunde Higher Institute of Medical Techniques (Ref No 70/ISTM–N/SGAC/2017), Bunia, DRC. Permission for field work was obtained from the Ituri Provincial Health Division (Ref. 054/433/DPS/IT/06/2016 and Ref. 054/472/DPS/IT/06/2017) and from all relevant health districts. Prior to enrolment, the objectives and procedures were explained to patients in the local language. Written informed consent was obtained and included permissions for taking photographs and publishing anonymized data. All participants diagnosed with an *S. mansoni* infection were treated with praziquantel (40mg/kg) [141]. All participants received Mebendazole (500mg, single dose, Vermox®) for general deworming, in accordance with the DRC's national deworming guidelines. Patients were referred to the district hospital for further follow-up.

Study area

Ituri province is situated in northeastern DRC and has a surface area of 65,658 km². With its capital in Bunia, Ituri is divided into five counties (territories) and 36 health districts. About 5.3 to 9.0 [307] million people of Sudanese, Nilotic, Bantu, Nilo-Hamite, and Pygmy ethnicities live in Ituri province.

For more than two decades, Ituri province has been subject to war, turmoil and social conflict. The socioeconomic situation in Ituri province is challenging, with a high degree of poverty and precarity. The DRC ranked 176/188 in the 2017 Human Development Index [308]. In 2011, a Water and Sanitation Program (WSP) strategic overview estimated that 50 million Congolese (75.0%) did not have access to safe water, while approximately 80–90% did not have access to improved sanitation [309]. Likewise, the UNICEF/WHO [310] 2017 database showed that in 2015, 84% of the DRC's rural population had no hygiene

facility, 45.3% had unimproved sanitation, 10.2% resorted to open defecation, 53% used unimproved water sources, and 16.0% used surface water.

The patients we report on here originate from four villages: Gupe and Ndaro, in the Angumu health district located on the shore of Lake Albert, east of Bunia; and Mandima and Pekele, in the Mandima and Lolwa health districts, respectively. The latter two villages are situated opposite each other, on the shores of a local river in southern Ituri province, approx. 160 km west of Bunia and approx. 200km from Lake Albert. *S. mansoni* transmission around Lake Albert has been documented since colonial times [118]. A recent study documented intensive transmission of *S. mansoni* in the villages around the lake [362] as well as in Mandima and Pekele.

The four villages were part of a large in-depth study of morbidity due to *S. mansoni* infection. The complete results of the study are available elsewhere [363]. Table 7.1 is an excerpt from the study, describing the epidemiological context of the four villages in which the severe cases resided. The villages are characterized by very high *S. mansoni* prevalence (72.4%, as assessed with a Kato-Katz test alone and 87.1% when Kato-Katz results were combined with point-of-care circulating cathodic *S. mansoni* antigen (POC-CCA) test results) and a high-intensity infection burden (16.0% had a heavy-intensity infection). Soil-transmitted helminths were almost absent, indicating that regular deworming with bendemidazoles had been conducted. More than half (56.4%) of the population was underweight. Intestinal morbidity (e.g. 60.1% with abdominal pain) and hepatosplenic morbidity was very frequent (e.g. 75.5% with splenomegaly, 71.2% with abnormal liver parenchyma).

Patient identification and assessment

Patients were identified during a community-based survey conducted between June and September of 2017. One patient presented at the Anguma hospital. The patients were clinically assessed and interviewed. Their general health concerns and history was assessed. An abdominal ultrasound was performed and a stool sample was examined for helminth infections.

Clinical examination

All participants underwent a clinical (physical) examination and a questionnaire-guided interview, both of which were conducted by an experienced physician with assistance from an experienced nurse.

The individual questionnaire focused on demographic, anthropometric, occupational, educational and religious characteristics, as well as on knowledge, attitude and practices related to *S. mansoni* infection and disease. It also included an assessment of signs and symptoms related to schistosomiasis, such as diarrhoea, blood in the stool in the two weeks preceding the study, and individual history of hematemesis at least once in their lifetime.

Abdominal ultrasound examination

An abdominal ultrasonographical examination was performed in accordance with the WHO/TDR guidelines [55], using a 1.0 Mhz probe U-Lite Sonoscanner Ultraportable HD Ultrasound Unit (U-Lite, Sonoscanner, 6, Rue André Voguet, Paris, France). A portable generator (MK, China) and solar powered batteries (for remote villages) were used as electricity sources.

Participants were examined in a supine position. The size of the left and right liver lobe, the portal vein diameter, and the length and width of the gall bladder were measured. Organ parenchyma was observed. Liver parenchyma patterns (Figure S7.1) were assessed following the WHO/TDR guidelines [55]. The length and width of the spleen were measured, and its texture determined.

Table 7.1: Schistosomiasis infection and morbidity in the four residential villages of the eight patients in 2017 (n=163).

Characteristics	N	%
Gender		
Females	86	52.8
Males	77	47.2
Age categories (years)		
6 – 9	32	19.6
10 – 14	40	24.5
15 – 29	36	22.1
30 – 39	22	13.5
≥40 – 49	33	20.2
Body mass index (kg/m² - categories)		
Overweight (≥25.0)	9	5.6
Normal weight (18.5-24.9)	62	38.0
Underweight (<18.5)	92	56.4
S. mansoni infection		
Kato-Katz test	118	72.4
POC-CCA test	129	79.1
KK+POC-CCA*	142	87.1
Infection intensity (KK only)		
Light	51	31.3
Moderate	41	25.2
Heavy	26	16.0
Soil transmitted helminths		
Trichuris trichiura	1	0.6
Ascaris lumbricoides	1	0.6
Hookworm	1	0.6
Clinical findings		
Diarrhoea	56	34.4
Blood in stool	56	34.4
Abdominal pain	98	60.1
Ultrasound findings		
Hepatomegaly	46	28.2
Splenomegaly	123	75.5
Pattern A	47	28.8
Pattern B/C	75	46.0
Pattern D/E	31	19.0
Pattern F	10	6.1

* KK+POC-CCA, combined any positive result (by Kato-Katz and/or by point-of-care circulating cathodic antigen (POC-CCA); KK only (Kato-Katz results only taken in account (at least one egg in at least 1 of 2 smears).

Parasitological examination

Participants were asked to provide one faecal sample (approx. 5 grams of morning stool) for a Kato-Katz test [79]. Labelled plastic containers were provided for collection. From each stool specimen, two thick smears of approximately 41.7 mg [79] were prepared and examined by experienced technicians. For hookworm assessment, the smears were examined by microscope within one hour after preparation. All slides were examined for *S. mansoni* within 24 hours. One third of the prepared smears were checked by the principal investigator. All recognized helminth eggs were counted and recorded for each species separately. The intensity of helminth infection was calculated by multiplying the mean number of eggs found on the two slides by 24. The result was expressed as eggs per gram (EPG) of stool [66]. *S. mansoni* infection intensities were classified as light (1–99 EPG), moderate (100–399 EPG), and heavy (≥400 EPG) [66].

Participants were asked to provide a urine sample (approx. 60 ml) for a point-of-care circulating cathodic antigen (POC-CCA) test. Pre-labelled wide-mouth plastic containers were provided for collection. The POC-CCA tests were performed according to the manufacturer's guidelines (Rapid Medical Diagnostics, Pretoria, South Africa) [364]. Urine was examined on the day of collection where possible. If the test was postponed until the next day, urine samples were kept at 2–8ºC in a solar fridge (Steca, Germany). A negative test result was declared if the POC-CCA band did not appear within 20 minutes. A positive result was recorded for any trace-, weak-, medium- and strong-coloured CCA bands. Questionable results were discussed among at least two technicians and the principal investigator. Both stool and urine examinations were performed at village health centre facilities.

Hemoglobin dosage

The hemoglobin rate was estimated at the Angunu hospital laboratory for two participants with extreme pallor of palpebral conjunctiva, using Sahli hemoglobinometer. Results were expressed as gram of total hemoglobin per deciliter of the blood (g/dl) [365].

7. 4 Descriptions of findings

During a field study of morbidity associated with *S. mansoni* infection, seven patients with severe intestinal and hepatosplenic disease were identified. One additional patient presented at the Angumu district hospital (patient #1). Table 7.1 gives an overview of the findings for eight patients with severe complications of schistosomiasis, including stool and urine examination results for *S. mansoni* infection, observed and reported symptoms, abdominal ultrasound examination results, treatment and outcomes.

The patients' ages ranged from 19 years to 57 years (mean 36 years); three patients were men. During examination, three patients reported an experience of hematemesis and for patient #1, it was the reason for hospitalization. Two patients were anaemic. All patients reported non-specific chronic abdominal (intestinal) symptoms, such as diarrhoea (five patients), abdominal pain and blood in the stool (four patients each) and weight loss (five patients).

Abdominal ultrasonography revealed ascites in four patients. All patients had portal hypertension with hepatosplenomegaly (six patients) or splenomegaly (two patients). Of the six patients for whom liver parenchyma patterns could be assessed, five displayed pattern D–E and one patient showed pattern B–C. The remaining two patients had severe ascites and the liver was hardly visible.

S. mansoni infection was confirmed for six patients. Patients #2 and #8 tested negative with POC-CCA and negative with Kato-Katz. Infection intensities ranged from 24 EPG to 2,832 EPG. One heavy-intensity infection was diagnosed, while two patients each had moderate- and light-intensity infections. All patients were treated with praziquantel (40 mg / kg BW). The hospitalized patient (patient #1) was discharged five days after treatment. One patient with severe ascites (patient #2) died two weeks after we saw her. Six patients were lost to follow-up.

Patient descriptions

Patient 1

A 31-year-old fisherman, residing in Gupe village on the shore of Lake Albert (Angumu health district), was urgently referred to Angumu hospital following five days of ongoing blackish feces and sudden vomiting of blood on the day of admission. He showed an altered general state and hippocratic facies. However, he was lucid and had an athletic constitution. He continued to vomit blood. He had a history of alcohol addiction but had never been treated for schistosomiasis.

His physical examination was unremarkable. Although the abdomen showed epigastric tenderness, its percussion revealed no matting and therefore, no ascites. The palpebral conjunctivae were pale and the bulbar conjunctivae anicteric. Examination of the thorax revealed tachycardia, but normal respiration. Abdominal ultrasound revealed hepatomegaly and splenomegaly. The liver was fibrotic and showed signs of portal hypertension (Figure 7.1.1).

He was severely anaemic (hemoglobin 7 g/dl). Direct microscopic examination of the brownish stool revealed the presence of *S. mansoni*'s side spur eggs (Figure 7.1.2). A thick smear preparation subject to the Kato-Katz technique showed severe infection intensity (2,832 EPG). The POC-CCA was positive. The malaria rapid diagnostic test was negative.

The patient was treated with praziquantel. In addition, saline infusion (NaCl 9‰, 1L) was administered, followed by 500ml of a 5% glucose solution with vitamin K. The patient received a blood transfusion and was discharged five days after admission.

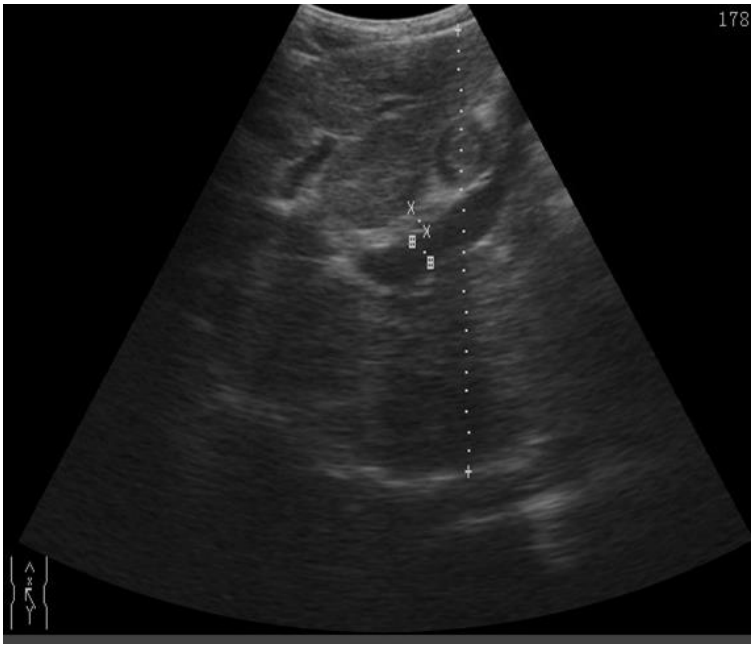

Figure 7.1.1. Ultrasound examination of patient 31 showing thickened periportal fibrosis and extended portal vein (portal hypertension) and granulomatous liver parenchyma.

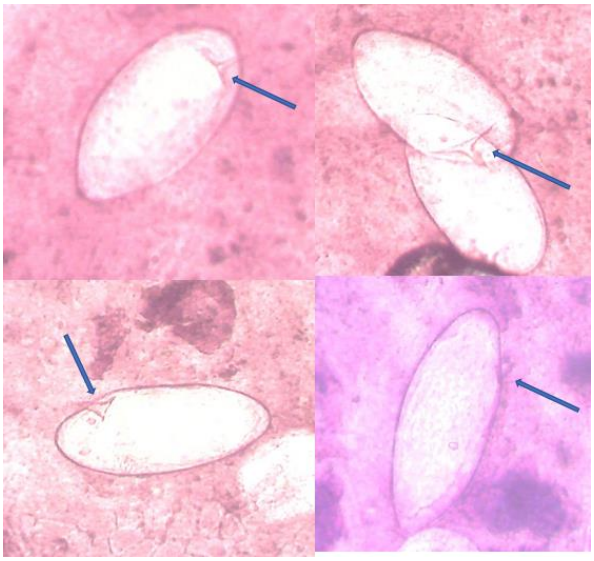

Figure 7.1.2. *Schistosoma mansoni* eggs in bloody stool of patient #1. Arrows show eggs' characteristic lateral spine.

Patient 2

A 57-year-old woman from Gupe village (Lake Albert, Angumu health district), presented with an extended abdomen. Her general state was severely altered. She had a history of schistosomiasis and had been treated several times. Her physical examination was typical of patients with severe intestinal schistosomiasis. She had epigastric tenderness of the abdomen; abdominal percussion revealed matting and extended ascites. She was severely anaemic (5g/dl/). She also had tachycardia, respiratory distress and tachypnoea due to the immense ascites.

The ultrasound examination revealed a highly cirrhotic and almost invisible liver, with extended ascites and splenomegaly (Figure 7.2).

Both stool and urine tests were negative for *S. mansoni* infection. Nevertheless, she received praziquantel treatment for possible chronic intestinal schistosomiasis complications. The patient died two weeks later.

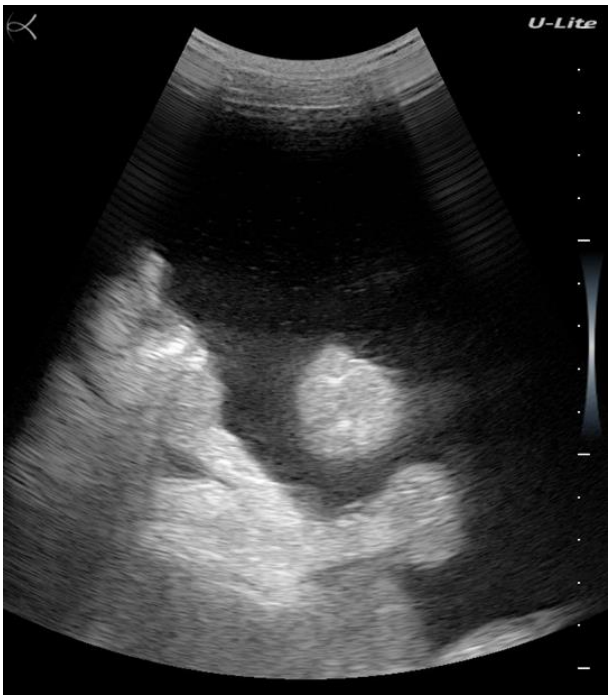

Figure 7.2. Ultrasound examination showing a generalized, end stage ascites of patient #2. The cirrhotic liver is hardly visible.

Patient 3

A 51-year-old fisherman from Gupe village (Lake Albert, Angumu health district) presented with an extended belly. His general state was severely altered (Figure 7.3). He was visibly tired, but was alert and lucid. He had a history of schistosomiasis and had been treated some years ago, several times. He had worked as a fisherman on Lake Albert for many years.

His abdomen showed epigastric tenderness; abdominal percussion revealed matting, indicating extended ascites. The ultrasound examination revealed a highly fibrotic and cirrhotic liver with massive ascites and splenomegaly (Figure 7.3).

The stool examination was negative for *S. mansoni* infection, but the POC-CCA was positive. The patient was treated with praziquantel. Three weeks after the visit, the patient's sister took him to his native village. He was subsequently lost to follow-up.

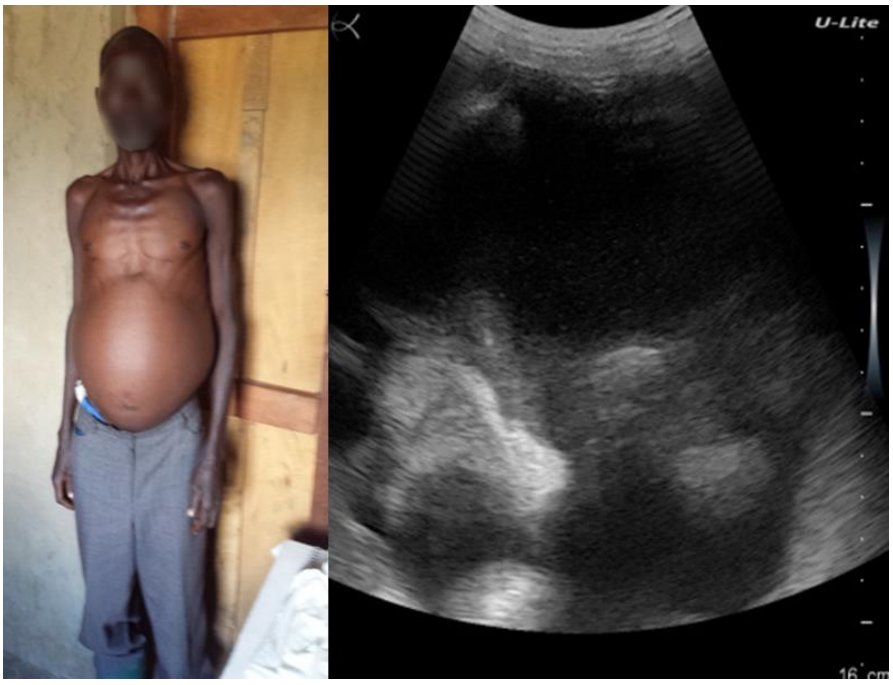

Figure 7.3. Patient #3 (left). Ultrasound examination (right) showing a generalized and end stage ascites. The cirrhotic liver was hardly invisible.

Patient 4

A 36-year-old man, residing in the fishing village of Ndaro, on the shore of Lake Albert, in Angumu health district, complained of abdominal pain, diarrhea and the presence of blood in the stool for some time. He had experienced hematemesis two times.

His general state was quite good (Figure 7.4). He had a history of schistosomiasis and had been treated two times. He had been working for several years as a fisherman.

His abdomen showed epigastric tenderness; however, abdominal percussion revealed no matting, thus no ascites. The ultrasound examination showed a highly fibrotic and cirrhotic liver with splenomegaly (Figure 7.4). Liver parenchyma was granulomatous and showed ruff and patches around the portal bifurcation (pattern D/E).

Both diagnostic tests were positive for a light-intensity *S. mansoni* infection (36 EPG). He was treated with praziquantel and subsequently lost to follow-up.

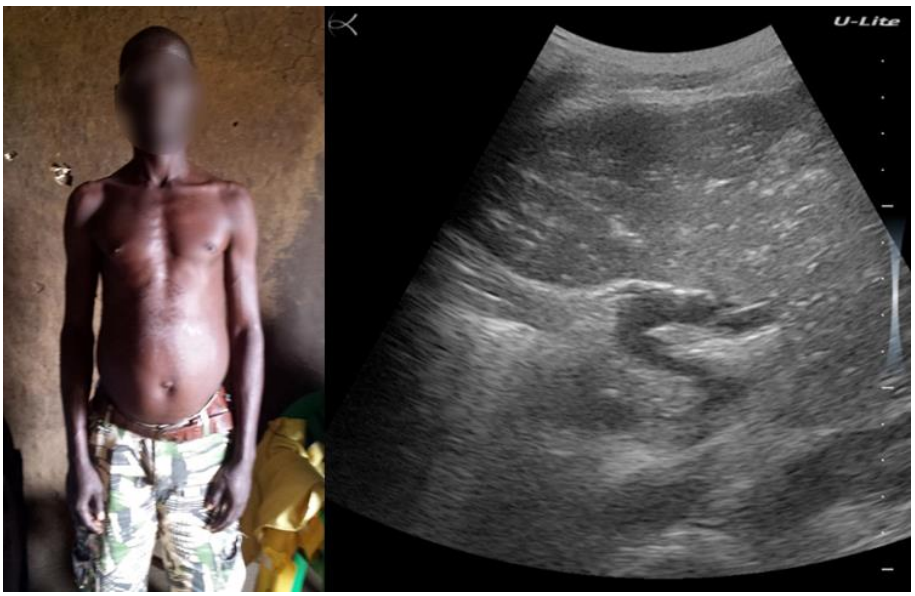

Figure 7.4. Patient #4 (left). Ultrasound examination (right) showing thick periportal fibrosis and granulomatous liver parenchyma.

Patient 5

A 22-year-old woman from Ndaro fishing village (Lake Albert, Angumu health district) complained of abdominal pain, diarrhea and the presence of blood in her stool for some time. She had vomitted blood on one occasion. She firmly believed that she was dealing with wizards.

She appeared exhausted. She could not recall having been treated before against schistosomiasis. She had been accompanying her husband on fishing trips before she became sick.

Her abdomen was extended (Figure 7.5) and showed epigastric tenderness; however, percussion revealed no matting, thus no ascites. The ultrasound examination showed a highly fibrotic liver, portal hypertension and splenomegaly (Figure 7.5). Liver parenchyma was granulomatous, showing ruff and patches around the portal bifurcation (pattern D/E).

Stool examination was positive for *S. mansoni* eggs, with a moderate-intensity infection (240 EPG); POC- CCA was also positive. She was treated with praziquantel and subsequently lost to follow-up.

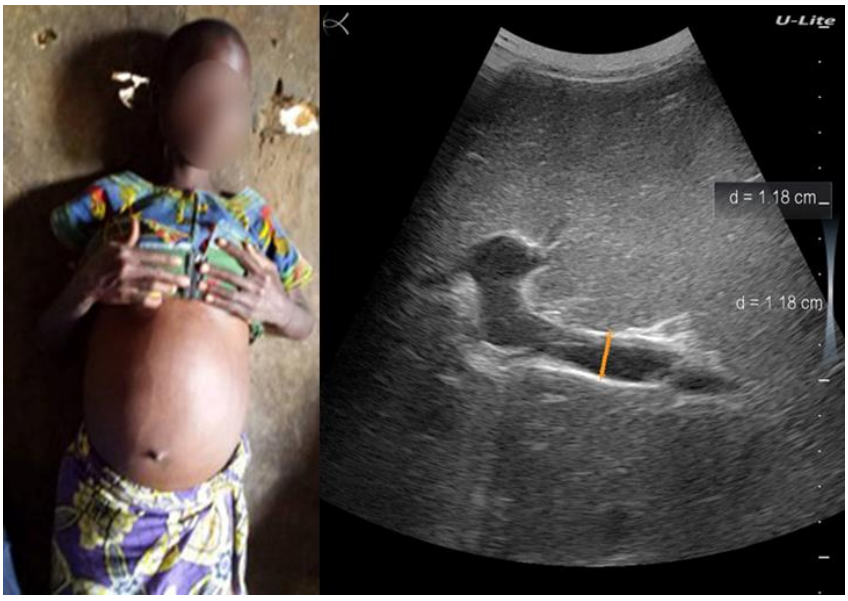

Figure 7.5. Patient #5 (left). Ultrasound examination (right) showed thickened periportal fibrosis and extended diameter of portal vein (orange bar).

Patient 6

A 19-year-old fisherman from Ndaro fishing village (Lake Albert, Angumu health district), complained of abdominal pain, diarrhea and the presence of blood in his stool. He had not vomitted blood. He reported severe weight loss, and appeared stunted and tired (Figure 7.6). He had a history of schistosomiasis and had been treated several times. He had been working as a fisherman already for many years.

His abdomen showed epigastric tenderness; however, percussion revealed no matting, thus no ascites. The ultrasound examination showed a highly fibrotic liver and splenomegaly. Liver parenchyma was granulomatous and showing ruff and patches around the portal bifurcation (pattern D/E).

He was diagnosed with a light-intensity *S. mansoni* infection. POC-CCA confirmed the infection. He was treated with praziquantel and subsequently lost to follow-up.

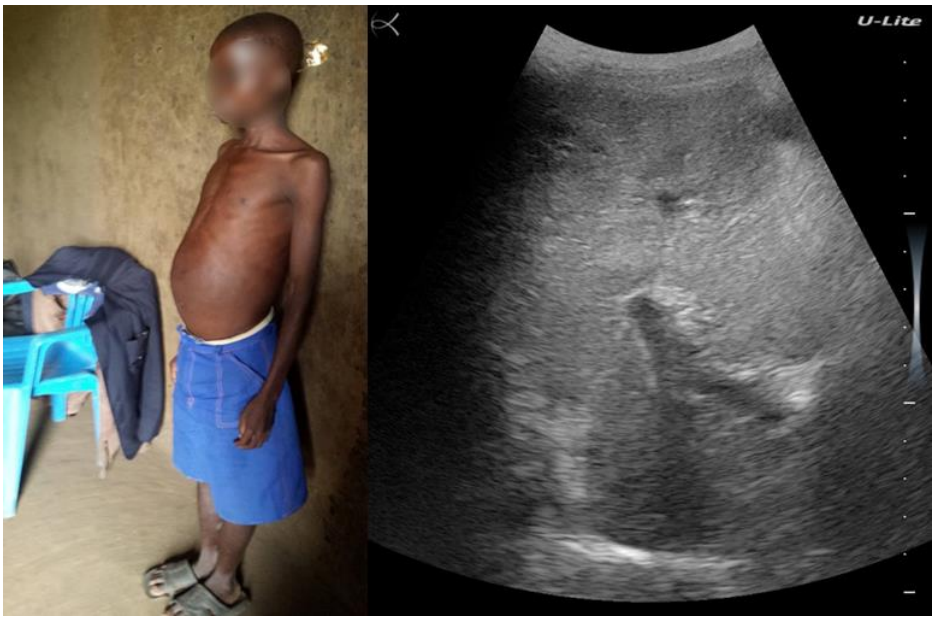

Figure 7.6. Patient #6 (left). Ultrasound examination (right) showing thickened periportal fibrosis and granulomatous liver parenchyma.

Patient 7

A 40-year-old farming woman, residing in the forest village of Mandima since birth (Mandima health district, southwestern Ituri), complained of weight loss, fatigue, abdominal pain and diarrhea, but no blood in the stool. She had not vomited blood. She had never heard of schistosomiasis and had never been diagnosed nor treated for it.

Her general state was bad. She had a very extended abdomen (Figure 7.7), which showed epigastric tenderness; percussion revealed matting, and thus ascites. The ultrasound examination showed a fibrotic liver with splenomegaly. Liver parenchyma was granulomatous, showing starry sky, ring and pipe-stem markings (pattern B/C), and portal hypertension.

The stool examination revealed a moderate-intensity *S. mansoni* infection (156 EPG). The POC-CCA was also positive. She received praziquantel treatment and was subsequently lost to follow-up.

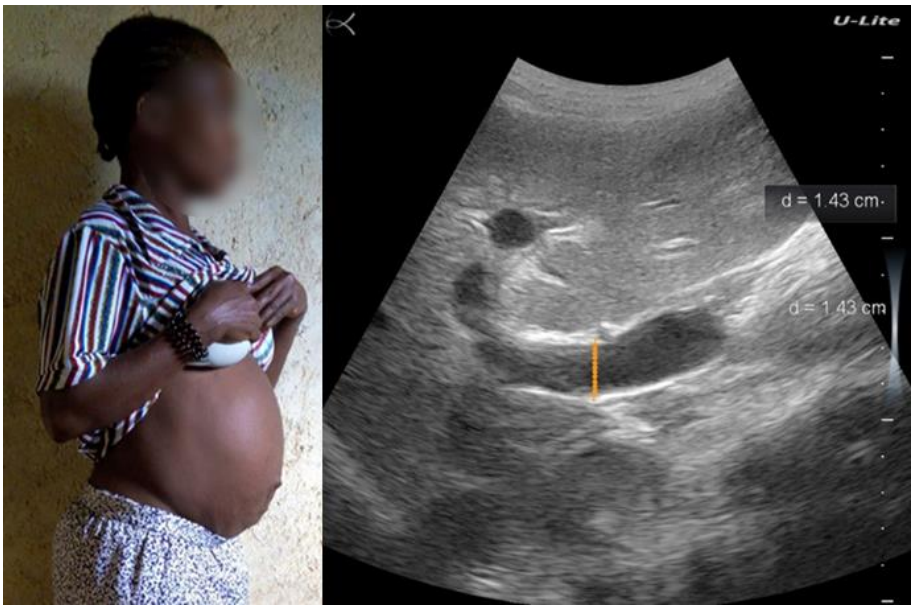

Figure 7.7. Patient #7 (left). Ultrasound examination (right) showing thickened periportal fibrosis and extended portal vein diameter (orange line, portal hypertension) and granulomatous liver parenchyma.

Patient 8

A 29-year-old farmer woman, residing in the forest village of Pekele (Lolwa health district, southwestern Ituri), a neighbouring village of Mandima, complained of weight loss, fatigue, abdominal pain and diarrhea, without blood in the stool. She had not vomitted blood before.

Her general state was bad, with a very extended belly (Figure 7.8). She had never heard of schistosomiasis and had not received treatment before.

Abdominal percussion showed dullness, revealing dramatic ascites. The ultrasound examination revealed hepatomegaly and splenomegaly. The liver parenchyma was granulomatous, showing starry sky (pattern B) markings.

Both diagnostic tests were negative for *Schistosoma* infection. She was treated with praziquantel and subsequently lost to follow-up.

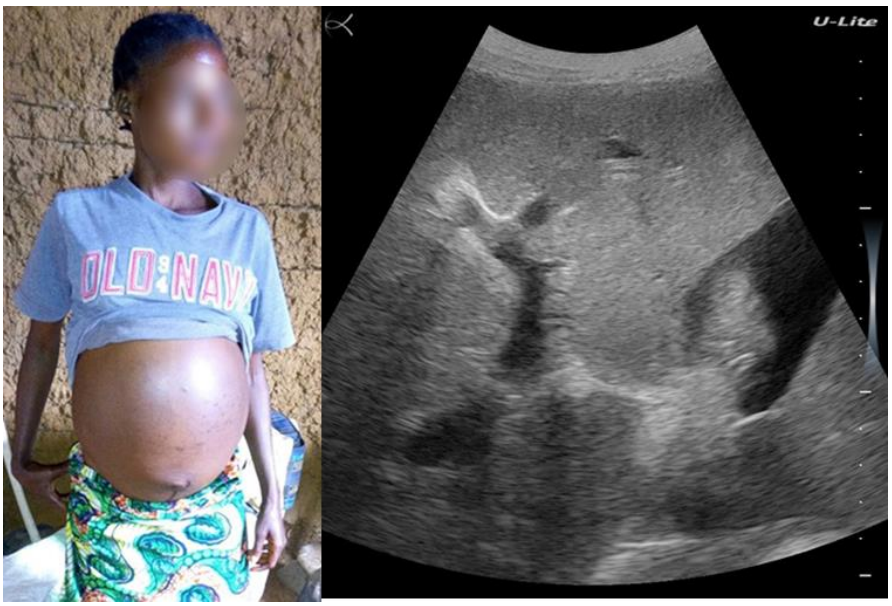

Figure 7.8. Patient #8 (left). Ultrasound examination (right) showing thickened periportal fibrosis, extended portal vein (portal hypertension) and granulomatous liver parenchyma.

7.5 Discussion

Intestinal schistosomiasis is highly prevalent in sub-Saharan Africa. Most infections occur in poor, rural communities where daily intensive, sometimes professional, contact with natural water bodies is unavoidable. These communities are characterized by a general lack of access to safe water, inadequate sanitation facilities and poor-quality health services that are often unable to diagnose and treat prevailing health concerns. It is generally accepted that intestinal schistosomiasis is associated with a considerable degree of morbidity and mortality, and thereby poses a substantial individual and public health burden. Yet, the extent of severe disease among members of the most neglected communities is rarely explored.

Our eight patients reported long-lasting intestinal symptoms consisting of recurring episodes of abdominal pain, diarrhea and blood in the stool. Three patients reported severe weight loss and general fatigue. Hepatosplenomegaly indicated portal hypertension, which was confirmed by ultrasonography for most patients. Three patients had one or several episodes of hematemesis. Four patients were diagnosed with ascites and advanced-stage liver fibrosis was observed in five patients. One patient died shortly after having been seen.

The clinical observations of our patients are typical of those with chronic schistosomiasis mansoni. Six of the eight patients were diagnosed with *S. mansoni*. We could not confirm an *S. mansoni* infection in two patients with ascites. Given the late stage of the disease of these patients, the finding is not surprising and has been reported before [366]. Furthermore, one patients was treated with praziquantel several times.

While our patients' clinical picture is consistent with schistosomiasis mansoni disease development, we cannot exclude other etiologies that might have contributed to the observed symptoms and complications. For example, intestinal protozoan infections are frequent in tropical Africa and are a major contributor to poor gastro-intestinal health. Viral hepatitis infections, which are of considerable prevalence in the DRC [355],of the may have also contributed to the morbidity observed. Likewise, *Plasmodium falciparum* is highly endemic to Ituri province [354] and could have contributed to the high prevalence

of splenomegaly observed among our patient group. As we could not assess all possible etiologies, there is a risk of overestimating the extent to which *S. mansoni* accounts for our patients' morbidity.

Three patients reported episodes of hematemesis. For patient #1, vomiting blood was the reason for his admission to hospital. While advanced schistosomiasis is one etiological factor for hematemesis, there are many others, such as gastric or duodenal ulcers, gastritis and esophangitis [359]. The use of non-steroidal, anti-inflammatory drugs was also identified as a contributing causal factor in a recent outbreak of hematemesis in Uganda [367]. However, among our patients, we diagnosed portal hypertension (enlarged portal vein diameter larger than 1.3 cm) and splenomegaly, both of which were found to be good predictors for active schistosomiasis as the etiological factor behind hematemesis [359].

Our patients resided in four villages that are highly endemic for *S. mansoni*. In a random sample (n=163) of individuals six-years and older in these villages, almost 90% (87.1%) tested positive for an *S. mansoni* infection, either by Kato-Katz technique and/or the rapid POC-CCA urine test (Table 7.1). One third of the villagers examined reported diarrhoea and/or blood in the stool. Upon abdominal ultrasound, more than one quarter were diagnosed with an enlarged liver, three quarters were diagnosed with splenomegaly and more than 70% had abnomal (fibrotic) liver parenchyma. The data demonstrate intensive transmission of *S. mansoni* and, hence, our eight patients are those with a most severe outcome — tip of the iceberg of large number of patients with morbidity in the community.

Six of our patients resided in two villages (Gupe and Ndaro) on the shore of Lake Albert, where the transmission of *S. mansoni* is well known and documented. The patients from these villages knew about schistosomiasis, and had previously been diagnosed and treated. The other two villages, Mandima and Pekele, are in southern Ituri and situated in a forested area. There, much less is known about the transmission of schistosomiasis. Neither of the two patients (patients #7 and #8) residing in these villages knew about schistosomiasis nor had they been diagnosed or treated before.

In DR Congo a lack of information exists on schistosomiasis and other neglected tropical diseases [13]. However, in 2016 and 2017 we have assessed *S. mansoni* infection and morbidity 59 villages. We found high infection prevalence levels, particularly on the shores of Lake Albert and in the forest region [362]. In latter area schistosomiasis is unknown. Indeed, during the colonial times, its prevalence was low (around 20%) in this area [118]. Today, it reaches record rates of more than 90% and hepatosplenomegaly of up 25% [362].

Indeed, in Ituri province ultrasound is a luxury in health services. They are not available in most hospitals, including some general hospitals and clinics. Yet, they are an indispensable diagnostic tool that can assess hepatosplenic morbidity guide the diagnosis and management of the patient.

7.6 Conclusions

Our clinical observations are limited to a few patients, which in turn limits the generalizability of our findings. Having said that, these cases document the severity of disease associated with *S. mansoni* infection in Ituri province. They provide further evidence for the severe degree of neglect of the Ituri population faces with regards to access to adequate water, sanitation and health services. The cases also present a strong argument for urgent, sound and targeted interventions.

Declarations

Ethical considerations and consent to participate

This study was approved by the Swiss Ethical Commission (Ref. No. UBE–15/78) and by the University of Kisangani's Research Ethical Commission (Ref No: CER/003/GEAK/2016). Research authorization was granted by the Nyankunde Higher Institute of Medical Techniques (Ref No 70/ISTM–N/SGAC/2017), Bunia, DRC. Permission for field work was obtained from the Ituri Provincial Health Division (Ref. 054/433/DPS/IT/06/2016 and Ref. 054/472/DPS/IT/06/2017) and from all relevant health districts. Prior to enrolment, the objectives and procedures were explained to patients in the local language. Written informed consent was obtained and included permissions for taking photographs and publishing anonymized data. All participants diagnosed with an *S. mansoni* infection were treated with praziquantel (40mg/kg) [141]. All participants received Mebendazole (500mg, single dose, Vermox®) for general deworming, in accordance with the DRC's national deworming guidelines. Patients were referred to the district hospital for further follow-up.

Consent to publication

Written informed consent was obtained and included permissions for taking photographs and publishing anonymized data.

Availability of data

Anonymized data can be obtained from the first authors upon reasonable request.

Competing interest

The authors have no competing interests to declare.

Funding

The study was funded by private funds (Poverty Foundation, Basel, Switzerland). The funders had no role in study design, data collection and data analysis, decision to publish, or preparation of the manuscript.

Author contributions

Conceptualization: MMN, PO, PH; Methodology: MMN, PO, DWN, GBS, PH; Investigation: MMN, DWN; Formal analysis: MMN, PO, GBS, PH; Writing - Original Draft: MMN, PO; Writing - Review & Editing: GBS, MB, PH; Supervision: MB, PH; Project administration and funding acquisition: PH.

Acknowledgements

We thank the Provincial Health Division officers, the Health District offices, the Institut Supérieur des Techniques Médicales de Nyankunde staff and the local authorities for their support. We thank Mrs Amena Briet for her efficient English editing and critical reading.

Authors' information

This section is not compulsory.

Supporting information captions

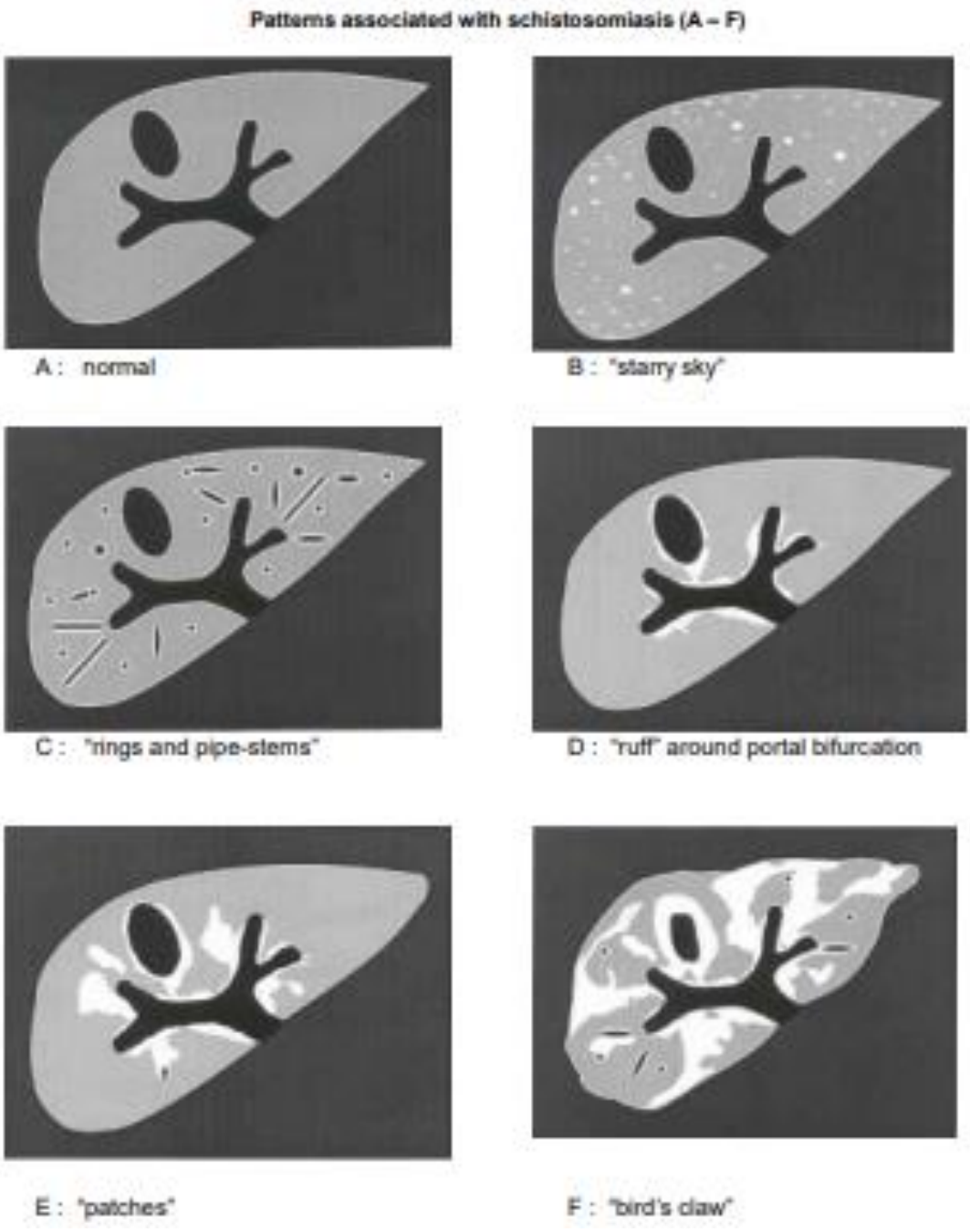

Figure S7.1: Liver image patterns associated with schistosomiasis, by [55].

Table 7.2: Medical information for 8 patients with severe schistosomiasis. Diagnosed in four purposively-selected villages in Ituri province.

Patient #	Age (years), Gender	Occupation	*Schistosoma mansoni* infection	Symptoms	Ultrasound	Treatment	Observations
1	31, male	Fisherman	Kato-Katz pos., severe infection intensity (2,832 EPG), POC-CCA pos.	Hematemesis, melena, anaemia, tachycardia, exhausted	Hepatosplenomegaly, portal hypertension, liver pattern D/E	PZQ, 40mg/kg	Cured 5 days later
2	57 female	House wife, fish trader	Kato-Katz and POC-CCA neg.	Anaemia, abdominal pain, mucus in stool, weight loss, tachycardia, dyspnoea, exhausted	Ascites, splenomegaly, liver almost invisible	PZQ, 40mg/kg	Died two weeks later
3	51, male	Fisherman	Kato-Katz neg.; POC-CCA pos.	Severe weight loss, abdominal pain, mucus in stool, tachycardia, exhausted	Ascites, splenomegaly, liver hardly visible, enlarged gall bladder and thick wall	PZQ, 40mg/kg	Lost to follow-up
4	36	Fisherman	Kato-Katz pos., light infection intensity (36 EPG); POC-CCA pos.	Hematemesis, abdominal pain, diarrhoea, blood in stools	Hepatosplenomegaly, highly fibrotic liver with portal hypertension, liver pattern D/E	PZQ, 40mg/kg	Lost to follow-up
5	22, female	House wife, fish trader	Kato-Katz pos., moderate infection intensity (240 EPG), POC-CCA pos.	Hematemesis, abdominal pain, diarrhoea, blood in stools, mucus in stool, exhausted	Hepatosplenomegaly, highly fibrotic liver with portal hypertension, liver pattern D/E	PZQ, 40mg/kg	Lost to follow-up
6	19, male	Fisherman	Kato-Katz pos., light infection intensity (24 EPG); POC-CCA pos.	Abdominal pain, diarrhoea, blood in stools, mucus in stool, severe weight loss, stunting, exhausted	Hepatosplenomegaly, highly fibrotic liver with portal hypertension, liver pattern D/E	PZQ, 40mg/kg	Lost to follow-up
7	40, female	House wife, farmer	Kato-Katz pos. moderate infection intensity (156 EPG); POC-CCA pos.	Abdominal pain, diarrhoea, blood in stools, severe weight loss, mucus in stool, exhausted	Ascites, hepatosplenomegaly, fibrotic liver with portal hypertension, liver pattern D/E, thick, irregular gall bladder wall	PZQ, 40mg/kg	Lost to follow-up
8	29, female	House wife	Kato-Katz and POC-CCA neg.	Abdominal pain, diarrhoea, blood in stools, mucus in stool, severe weight loss, exhausted	Ascites, hepatosplenomegaly, fibrotic liver with portal hypertension, liver pattern B/C, enlarged gall bladder	PZQ, 40mg/kg	Lost to follow-up

Pos. positive, neg. negative, POC-CCA point-of-care circulating cathodic S. mansoni antigen test

8 General discussion

8.1 Summary

This study is the largest and most in-depth analysis of schistosomiasis in eastern DR Congo since the colonial times, a region where wars and continued armed conflicts, mass population displacements and extreme poverty are a fertile ground for spread and perpetuation of various infectious diseases. This chapter recalls the highlights of our study and lays the groundwork for future activities to eradicate schistosomiasis in the Ituri province. We have shown that Ituri province, the location of the first description of schistomiasis in 1926, remains one of the heaviest foci of schistosomiasis mansoni in the world in terms of prevalence and morbidity. Prevalence and intensity of *S. mansoni* infection are very high almost everywhere in the province. Morbidity is also high. Children are the most frequent victims and they carry the heaviest burden of infection. There is every reason to believe that everyone is at risk of *S. mansoni* infection, especially since all the social strata of the population are affected. It is therefore clear that disease control and eradication activities need to involve the entire population. To do otherwise would risk perpetuating *S. mansoni* infection in the province. The overall picture of morbidity is more subtle. In this region haunted by all sorts of endemics and multi-faceted epidemics, any hasty interpretation might be questionable. Characteristic signs and symptoms such as abdominal pain, diarrhoea or blood in stool are commonly reported. Intestinal worms, amoebiasis, shigellosis, cholera, to name but a few, are all possible causes. In addition to highly endemic malaria, diseases and conditions such as yellow fever, viral hepatitis, sickling anaemia, chemical pollution related to mining, frequent consumption of local prepared impure alcohol, and many other causes can contribute to liver fibrosis, hepatomegaly and splenomegaly. The association between organomegaly and *S. mansoni* infection has been disputed. Precise diagnostic tools are therefore needed. We show here the limitations of the current clinical standard, the Kato-Katz technique, in terms of sensitivity. The circulating cathodic antigen (CCA) test showed to be more sensitive and can contribute greatly in improving the detection of *S. mansoni* infection. Also, the cost of testing individuals and communities with this tool remains high. In a contrasting and complex region like Ituri province, it is important to combine several control measures. To treat active infection, in particular high-intensity infection that comes with high morbidity is as important as breaking the reinfection cycle with adequate control measures including health education, sanitation, improved water supply, and hygiene, environmental measure like snail control will be most

promising if performed in parallel. This will have the double advantage of firstly reducing the morbidity, therefore the suffering of the population, and also reducing the transmission of the infection. We also plan to launch self-care activities for the population by working closely with different stakeholders of the public and socioeconomic sectors, and with civil society, micro-credit and mutual health organizations. These activities will go a long way toward empowering individuals and households in health care. However, all these measures will be difficult to implement as long as the security situation remains deleterious across the province. Thus, the provincial and national governments will remain the main support in this regard.

8.2 Result interpretation

The study reveals that, in the Ituri province, current *S. mansoni* prevalence is much higher than in colonial times [118] and higher than in other parts of the country [53, 62, 68, 126, 127]. The differences in prevalence rates between the colonial period and the present day are tens of percent, with the exception of one region, the shore of Lake Albert, where it just remains very high. For example, in the Mambasa county, prevalence rates were around 20% [118], but today they reach 90% or more. This cannot be explained only by availability of better diagnostic tools compared to the colonial era, when direct wet smear was used and may have had lower sensitivity: for example, for the county of Aru at the northern of the province, the differences are much smaller when we compare results obtained with Kato-Katz with those of colonial times. The levels of *S. mansoni* infection that we found in our study are comparable with those reported by Kabatereine and by Tukahebwa, two high-prevalence locations in Uganda [30, 52]. They are alarming and confirm that schistosomiasis is highly endemic in entire Ituri, to a degree that is similar to situations historically seen only in fishermen villages by the lake.

Our results show little difference between male and female. In younger age, both girls and boys show comparable trends of infection. But globally, men were more infected than women. This situation may be explained by the fact that men, through their responsibility of taking care of the family are more likely to work more in contact with water than women. Most of studies in literature find that men are the most infected [30, 52, 60, 63]. However, among school-aged children, girls were more infected than boys in our data. In Ituri, this is understandable because one observes that girls (as women in general) often spend more time than boys in contact with

water: Girls always do the work of cleansing the dishes or washing clothes, most often in surface waters. Meanwhile, boys spend more time playing.

We found many differences in prevalence rates among age groups. Striking results were found in children under five, where prevalence exceeded 60%. Although many authors agree that schistosomiasis is more and more common in infants [368, 369], such rates show clearly that schistosomiasis mansoni is deeply rooted in the region. The age dependent trend of the infection is typical of those in endemic areas where there is no control program [349]. However, we observed that the parameters fell slowly in women. This calls for further investigations for understanding the cause. Among school-aged children (5 – 14 years) high levels of both *S. mansoni* infection prevalence and intensity were surprising as they were even seen if formally, a control programs in some areas exists [132]. This situation seems paradoxical but might arise if a control program is not adapted to the prevalence in a community, in particular if rapid reinfection is frequent because the infection cycle at the community level is not interrupted. Without a thorough surveillance program, it will be difficult to understand the main reasons in detail. Much of the literature on this subject abounds in the same sense that this age group is the most affected [52]. Ituri is no exception in this matter.

Significant differences in infection prevalence and intensity were found among people from different health districts, villages, differing in rural/urban setting, occupation, and ethnic belonging backgrounds. However, the main risk factors that endanger individuals were poverty, individual and community behaviours, contact with water, and the lack of the knowledge of disease transmission and prevention. Lake Albert is well known as the cradle of schistosomiasis mansoni in Ituri since colonial time. The disease was rife at the edge of Lake Albert and locations that are situated along the shore of the lake; its fisheries, including Mahagi-Port, Ndaro and Kasenyi were then deemed infamous as frequency of cases was high [118]. Our study confirmed that this situation still persists today, nearly 100 years after description of the problem. All the visited villages along the lake such as Kadjugi, Kalako, Kalambo, Mita, Ndaro, Ndawe, Jupamuswa, Jupanyarabi, Paluo, Ramogi, Gupe, Pamaya, Wikidhi, and Kolokoto presented very high infection prevalence and intensity. However, due to population displacements related to war, ethnic conflicts and mining, many people formerly living in this region have migrated to the southern and western forest regions. Naturally, the disease has also moved with the displaced. So, it is not surprising that we now have the highest infection prevalence and intensity in this region. Made up of numerous swamps and almost permanent

humidity, villages such as Pekele, Mambau, and Mandima have just "won the monopoly" of high infection prevalence in the province.

Our study also showed that infection prevalence and intensity is typically higher in rural areas than in urban ones. This does not mean that rural populations are more susceptible to *S. mansoni* infection than those living in city. We simply think that they come into contact with water more frequently than the latter. Risky activities such as farming, fishing, and hunting which involve frequent contact with water are more practiced in rural areas. However, other water activities including washing clothes and dishes, washing motorcycles and cars, bathing, swimming, playing in water are similarly practiced in the city as well as in rural areas, which puts the two populations on an equal footing. Thus, the inhabitants of the urban villages of Ngezi, Sukisa, and Kindia in the city of Bunia even run a higher risk of infection than those of Gupe and Kadjugi, historical high-risk locations at the shore of Lake Albert.

We also have no evidence to suggest that populations of one or another ethnic group are more susceptible to *S. mansoni* infection than others. However, we have noted that the Alur population, living at the shore of Lake Albert and whose men are known to be fearless fishermen had the highest level of *S. mansoni* infection intensity. In addition, Pygmies were among the people with the highest rates for both *S. mansoni*, *Trichuris trichiura*, and *Ascaris lumbricoides* infection prevalence. Here again, we doubt their higher susceptibility to contracting the infections. It is probably the environment in which they live, characterized with high humidity in the forest, extreme poverty, lack of both sanitation and water supply, and their life essentially of hunting, fishing and gathering in the forest which expose them more to these infections.

Several explanations can be provided concerning these alarming rates of *S. mansoni* infection in the region. In the first place, poverty. Many researchers have shown that schistosomiasis is a poverty-related disease [39]. Poverty pushes people to take thoughtless risks. Poverty conditions people to relegate their health to a low priority. Poverty is deeply rooted in the post-conflict DR Congo. Extreme poverty is found almost everywhere in the Ituri province as Ituri was plagued with war, tribal conflicts over two decades [253]. Thus, one can understand why, even today, schistosomiasis is so prevalent in our province. Indeed, the results we have found sufficiently prove that the population of the province of Ituri is impoverished and therefore given up to any kind of biological attack, including that of parasites such as *S. mansoni*. When we find such high prevalence even among children under five, this is sufficient proof that the magnitude of the problem is major.

However, poverty is not solely responsible for this alarming situation. The education and behaviour of people are also important. What prevents a housekeeper from digging and building a latrine and making sure that each member of the family uses it? Nothing but the lack of good will or knowledge. Our results show that a significant portion of households lack latrines and a significant portion of individuals do not use latrines. These households and individuals were found to be significantly more likely to be infected than others. In addition, few people know about the disease, its transmission and prevention. However, those who had better knowledge were the most infected. What a paradox. However, since colonial time, it was already observed that the imposed latrines were kept spotlessly clean, but the banks of the nearby streams were covered with human excrement. We observed the same situation during our study, especially in the fishing villages. Instead of emptying into the latrine hole, it was on the floor and around the latrine that excrement was spread. Kabatereine et al. [30] also made the same observations when working in a fishing village in neighbouring Uganda. However, even if education is an ungrateful and time-consuming work, it must be done [118].

Colonial health officials undertook regular investigations in Ituri to detect new foci across the province. The different reports clearly determined the foci and regions according to the respective levels of prevalence and risk of infection. These investigations were carried out in schools, mining camps and health facilities. However, the entire area bordering the lake concentrated most of the control efforts. However, in that time, all cases diagnosed in health facilities and during investigations were systematically treated. Measures to restrict the movement of population were taken, regularly monitored and verified. The general trend was towards a progressive decrease in the incidence and prevalence of the disease. Nevertheless, since the independence of the country, the disease is mentioned only in the global reports of the care services. No major action has been specifically undertaken to detect and control the disease. People have little knowledge about the disease. Thus, in the 1970s and 1980s, for example, a common and prevalent paradigm within the population made schistosomiasis “a disease of people from the lake”, of fishermen essentially and maybe related to the consumption of lake water. Ascites, for instance, was commonly considered as consequence of the consumption water of the lake. In this way, it sadly became as a joke to tackle somebody with big belly with the word “*bilarizozi*”, the local term for bilharziasis. Thus, many people who went to the lakeshore took good care not to consume the water of the lake, even if they could wash themselves there. This region haunted by several epidemic, control measures are of great value.

Control of schistosomiasis is the biggest challenge in this vast region of Ituri. Education for behaviour change is the top priority. However, it has been an ungrateful and time-consuming work [118]. The Congolese people in general, and Iturian in particular, is fond of audio-visual information accompanied by music. A strategy that exploits radio as well as advertising pamphlets, and films targeting children could bring good results.

Alternatives of biological control based on the introduction of predatory such as molluscivorous crabs and fish has been advocated since several years. Inserting crab species such as *Potamon didieri* and *Potamon lirrangese* were successfully explored as control measure, alongside with *Xenopus laevis victorianus*, *Serranochromis macrocephala* and *Chrysichtys mabusi* fish species. *Chrysichtys mabusi* fish was found to be strictly mollusciphage [118]. Recently, some authors proposed prawn aquaculture for both poverty alleviation and schistosomiasis control [119]. Such initiatives are to be promoted and expanded.

If this is the situation in the Ituri, what about the neighbouring provinces and the rest of the country? The few available publications show that schistosomiasis mansoni, although in different degrees, occurs widely and remains a public health concern in DRC. Recurrent and massive infections can result in important morbidity. When it is not diagnosed and treated, the large proportion of the infected individuals will remain a reservoir from which infection will be continually spread and perpetuated.

DRC is maybe the country who bear the highest burden of intestinal schistosomiasis. However, this burden remains unknown and the integrated control program is not up to the challenge.

Since the colonialists left DRC in 1960, no specific study of this size has ever been undertaken in this part of the country. For the first time in decades, our study has thus established that schistosomiasis is a real public health issue in Ituri province. Prevalence has reached alarming levels, with rates exceeding 90% in some villages. In addition, *S. mansoni* infection affects mainly children. Projected to the province population, this means that millions of undiagnosed and, moreover, untreated people live in the region.

Furthermore, morbidity was found to be high. Yet, children always pay a heavy price. Their growth and their school education are likely to suffer. This presages a dark future for their future contribution as adult citizens to the development of their communities, on the one hand, and the country on the other.

9 Conclusions, Implications, Activities, Policy

In conclusion, this thesis shows that schistosomiasis is a huge health problem in Ituri region, is responsible for significant morbidity and afflicts a majority of the population. It is caused by a water-borne parasite but facilitated, perpetuated and potentiated by the wars and ongoing armed conflicts in the region, by the profound poverty of the population despite the wealth of natural and mineral resources of the area. Schistosomiasis affects more than 3 million inhabitants in the Ituri province only, compared to Ebola that killed 2000 persons in the entire DR Congo in the last few years but bound most healthcare efforts, and a measles epidemy that involved 250'000 in the entire eastern part of DR Congo.

Treating individual with specific diseases is not the same as improving healthcare, and the findings show that an approach to improve healthcare must be comprehensive: establish and preserve peace, educate people, overcome poverty, use the richness of natural resources to improve living conditions, improve infrastructure, train healthcare personnel and assure healthcare personnel salaries, combat corruption. Then, plan and perform campaigns that encompass an entire spectrum of health threats, using mass drug treatment, education, vaccination, early warning systems, vector control and individual preventive measures. Understand potential and limitations of diagnostic tests and use them where they bring best results, for example Kato-Katz tests as a well suited instrument to extrapolate village-wide prevalence and infection severity, in combination with point-of-care antigen testing as a more expensive test more suited to better detect individual disease in a low-prevalence environment and for monitoring of control programs near to eradication. The scope of schistosomiasis calls for concerted efforts of many players: the country government, provincial government, educational institutions, hospitals, ambulatory health care providers, infrastructure developers, industrial mining companies and artisanal miners, non-profit organizations, population-based initiatives like micro-credit organizations, mutual health insurances, youth organizations of the DRC to ensure the peace and well-being of its population.

The large unmet needs and the current failure to overcome this poverty disease also call for novel, innovative, unconventional and holistic approaches covering education, societal and technological means. What role can we expect from digitalization, artificial intelligence, nanotechnology-based diagnostic tools and therapeutic approaches, robotics, wireless technologies ? What technologies currently evolving in developed countries can be applied with great benefit for mankind to such poverty diseases ? What role will a social group based

approach, e.g. mass treating of families and villages, play in the future, compared to an individualized approach in the upcoming era of personalized medicine ?

There are great challenges, but also historic opportunities: the extremely high prevalence allows to reach a large number of affected persons with limited effort and resources. In heavily infected population segments, individual therapy may not be needed and may not be cost-effective and comprehensive therapy of families, villages, districts may be a simple strategy allowing to help many persons even with a moderate budget. While the issue of the endemicity of schistosomiasis and the burden it represents in sub-Saharan Africa may not be new, given the particular situation of the DRC and the WHO's stated desire to see this disease is eradicated in the world, the issue of schistosomiasis in Ituri is of an urgent nature. The eradication of schistosomiasis is a long-term goal. Despite the difficulties in view, the first steps should be taken. However, the desire to do so will have to be the main leitmotiv of anyone who gets started.

10 References

1. Smith, J. and E.M. Taylor, *What Is Next for NTDs in the Era of the Sustainable Development Goals?* Plos Neglected Tropical Diseases, 2016. **10**(7).
2. *Neglected tropical diseases: becoming less neglected.* Lancet, 2014. **383**(9925): p. 1269-1269.
3. *Control of neglected tropical diseases: the need to integrate knowledge, social, and political processes.* Asian Biomedicine, 2014. **8**(6): p. 697-698.
4. *Neglected tropical diseases: no longer someone else's problem.* Lancet Infectious Diseases, 2014. **14**(10): p. 899-899.
5. Molyneux, D.H., *Neglected tropical diseases: now more than just 'other diseases' - the post-2015 agenda.* International Health, 2014. **6**(3): p. 172-180.
6. Mitra, A.K. and A.R. Mawson, *Neglected Tropical Diseases: Epidemiology and Global Burden.* Trop Med Infect Dis, 2017. **2**(3).
7. Hotez, P.J. and A. Kamath, *Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden.* PLoS Negl Trop Dis, 2009. **3**(8): p. e412.
8. Lumbala, C., et al., *Human African trypanosomiasis in the Democratic Republic of the Congo: disease distribution and risk.* Int J Health Geogr, 2015. **14**: p. 20.
9. Rock, K.S., et al., *Quantitative evaluation of the strategy to eliminate human African trypanosomiasis in the Democratic Republic of Congo.* Parasit Vectors, 2015. **8**: p. 532.
10. Opoku, N.O., et al., *Single dose moxidectin versus ivermectin for Onchocerca volvulus infection in Ghana, Liberia, and the Democratic Republic of the Congo: a randomised, controlled, double-blind phase 3 trial.* Lancet, 2018. **392**(10154): p. 1207-1216.
11. Bakajika, D.K., et al., *Filarial Antigenemia and Loa loa Night Blood Microfilaremia in an Area Without Bancroftian Filariasis in the Democratic Republic of Congo.* American Journal of Tropical Medicine and Hygiene, 2014. **91**(6): p. 1142-1148.
12. Wanji, S., et al., *Validation of the rapid assessment procedure for loiasis (RAPLOA) in the democratic republic of CongoS.* Parasites & Vectors, 2012. **5**.
13. Rimoin, A.W. and P.J. Hotez, *NTDs in the Heart of Darkness: The Democratic Republic of Congo's Unknown Burden of Neglected Tropical Diseases.* Plos Neglected Tropical Diseases, 2013. **7**(7).
14. WHO, *Schistosomiasis and soil-transmitted helminth infections, WHA 54.19 resolution.* 2001.
15. WHO, *WHA 65.21 Elimination of schistosomiasis.* 2012.
16. Who, *Sustaining the Drive to Overcome the Global Impact of Neglected Tropical Diseases: Second WHO Report on Neglected Tropical Diseases.* Sustaining the Drive to Overcome the Global Impact of Neglected Tropical Diseases: Second Who Report on Neglected Tropical Diseases, 2013: p. 1-138.
17. Molyneux, D., *Neglected tropical diseases.* Community Eye Health, 2013. **26**(82): p. 4.
18. WHO, *WHA 66.12 Neglected tropical diseases.* 2013.
19. Hotez, P.J., *Ten Global "Hotspots" for the Neglected Tropical Diseases.* Plos Neglected Tropical Diseases, 2014. **8**(5).
20. Hotez, P.J., et al., *Innovation for the 'Bottom 100 Million': Eliminating Neglected Tropical Diseases in the Americas.* Hot Topics in Infection and Immunity in Children Ix, 2013. **764**: p. 1-12.
21. van Lieshout, L. and M. Yazdanbakhsh, *Landscape of neglected tropical diseases: getting it right.* Lancet Infectious Diseases, 2013. **13**(6): p. 469-470.
22. Deribe, K., et al., *Stigma towards a Neglected Tropical Disease: Felt and enacted Stigma Scores among Podoconiosis Patients in Northern Ethiopia.* Bmc Public Health, 2013. **13**.
23. Dimitri, N., *Global burden and investment for neglected diseases?* Lancet Infectious Diseases, 2010. **10**(10): p. 658-660.
24. Loker, E.S. and B.V. Hofkin, *An Introduction to Parasitism.* Parasitology: A Conceptual Approach, 2015: p. 1-20.
25. Loker, E.S. and B.V. Hofkin, *Parasitology: A Conceptual Approach.* Parasitology: A Conceptual Approach, 2015: p. 1-560.

26. Loker, E.S. and B.V. Hofkin, *An Overview of Parasite Diversity.* Parasitology: A Conceptual Approach, 2015: p. 21-66.
27. Loker, E.S. and B.V. Hofkin, *The Parasite's Way of Life.* Parasitology: A Conceptual Approach, 2015: p. 67-109.
28. Lawton, S.P., et al., *Genomes and geography: genomic insights into the evolution and phylogeography of the genus Schistosoma.* Parasites & Vectors, 2011. **4**.
29. Kabatereine, N.B., et al., *Epidemiology and geography of Schistosoma mansoni in Uganda: implications for planning control.* Tropical Medicine & International Health, 2004. **9**(3): p. 372-380.
30. Kabatereine, N.B., et al., *Epidemiology and morbidity of Schistosoma mansoni infection in a fishing community along Lake Albert in Uganda.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2004. **98**(12): p. 711-718.
31. Kajihara, N. and K. Hirayama, *The War against a Regional Disease in Japan A History of the Eradication of Schistosomiasis japonica.* Trop Med Health, 2011. **39**(1 Suppl 1): p. 3-44.
32. Standley, C.J., et al., *Zoonotic schistosomiasis in non-human primates: past, present and future activities at the human-wildlife interface in Africa.* Journal of Helminthology, 2012. **86**(2): p. 131-140.
33. Silva, L.L., et al., *The Schistosoma mansoni phylome: using evolutionary genomics to gain insight into a parasite's biology.* Bmc Genomics, 2012. **13**.
34. Webster, J.P., C.M. Gower, and A.J. Norton, *Evolutionary concepts in predicting and evaluating the impact of mass chemotherapy schistosomiasis control programmes on parasites and their hosts.* Evolutionary Applications, 2008. **1**(1): p. 66-83.
35. WHO, *Schistosomiasis. Key facts.* 2019.
36. Steinauer, M.L., M.S. Blouin, and C.D. Criscione, *Applying evolutionary genetics to schistosome epidemiology.* Infect Genet Evol, 2010. **10**(4): p. 433-43.
37. Di Bella, S., et al., *History of schistosomiasis (bilharziasis) in humans: from Egyptian medical papyri to molecular biology on mummies.* Pathog Glob Health, 2018. **112**(5): p. 268-273.
38. Ross, A.G.P., et al., *A new global strategy for the elimination of schistosomiasis.* International Journal of Infectious Diseases, 2017. **54**: p. 130-137.
39. Tchuente, L.A.T., et al., *Moving from control to elimination of schistosomiasis in sub-Saharan Africa: time to change and adapt strategies.* Infectious Diseases of Poverty, 2017. **6**.
40. Savioli, L., et al., *Building a global schistosomiasis alliance: an opportunity to join forces to fight inequality and rural poverty.* Infectious Diseases of Poverty, 2017. **6**.
41. WHO, *Status of schistosomiasis endemic coutries: 2017.* 2019.
42. CDC, *Schistosomiasis.* 2019.
43. Laboratory, D.B., *Guide de terrain des gastéropodes d'eau douce africains, 1: Afrique occidentale.* 1981.
44. Gray, D.J., et al., *CLINICAL REVIEW Diagnosis and management of schistosomiasis.* British Medical Journal, 2011. **342**.
45. Gryseels, B., et al., *Human schistosomiasis.* Lancet, 2006. **368**(9541): p. 1106-1118.
46. Loker, E.S. and B.V. Hofkin, *Parasite Versus Host: Pathology and Disease.* Parasitology: A Conceptual Approach, 2015: p. 159-192.
47. Tidy, C. *Schistosomiasis*. 2013; Available from: www.patient.co.uk/doctor/schistosomiasis-pro.
48. Colley, D.G. and W.E. Secor, *Immunology of human schistosomiasis.* Parasite Immunology, 2014. **36**(8): p. 347-357.
49. Li, Y.S., et al., *Severe hepatosplenic schistosomiasis: clinicopathologic study of 102 cases undergoing splenectomy.* Human Pathology, 2011. **42**(1): p. 111-119.
50. Li, Y.S., et al., *Oriental schistosomiasis with neurological complications: case report.* Annals of Clinical Microbiology and Antimicrobials, 2011. **10**.
51. Beck, L., et al., *Discriminating acute from chronic human schistosomiasis mansoni.* Acta Tropica, 2008. **108**(2-3): p. 229-233.
52. Tukahebwa, E.M., et al., *A Very High Infection Intensity of Schistosoma mansoni in a Ugandan Lake Victoria Fishing Community Is Required for Association with Highly Prevalent Organ Related Morbidity.* Plos Neglected Tropical Diseases, 2013. **7**(7).

53. Matangila, J., et al., *Malaria, schistosomiasis and soil transmitted helminth burden and their correlation with anemia in children attending primary schools in Kinshasa, The Democratic Republic of the Congo.* Tropical Medicine & International Health, 2015. **20**: p. 180-180.
54. Ibironke, O.A., et al., *Diagnosis of Schistosoma haematobium by Detection of Specific DNA Fragments from Filtered Urine Samples.* American Journal of Tropical Medicine and Hygiene, 2011. **84**(6): p. 998-1001.
55. Richter, J., et al., *Report of the second satellite symposium on ultrasound in schistosomiasis.* Memorias Do Instituto Oswaldo Cruz, 2001. **96**: p. 151-156.
56. Harter, G., et al., *Diagnosis of neuroschistosomiasis by antibody specificity index and semi-quantitative real-time PCR from cerebrospinal fluid and serum.* Journal of Medical Microbiology, 2014. **63**: p. 309-312.
57. Ferrari, T.C.A. and P.R.R. Moreira, *Neuroschistosomiasis: clinical symptoms and pathogenesis.* Lancet Neurology, 2011. **10**(9): p. 853-864.
58. Ferrari, T.C.A., P.R.R. Moreira, and A.S. Cunha, *Clinical characterization of neuroschistosomiasis due to Schistosoma mansoni and its treatment.* Acta Tropica, 2008. **108**(2-3): p. 89-97.
59. Davis, S.M., et al., *Morbidity associated with schistosomiasis before and after treatment in young children in Rusinga Island, western Kenya.* Am J Trop Med Hyg, 2015. **92**(5): p. 952-8.
60. Koroma, J.B., et al., *Geographical Distribution of Intestinal Schistosomiasis and Soil-Transmitted Helminthiasis and Preventive Chemotherapy Strategies in Sierra Leone.* Plos Neglected Tropical Diseases, 2010. **4**(11).
61. Midzi, N., et al., *Distribution of schistosomiasis and soil transmitted helminthiasis in Zimbabwe: towards a national plan of action for control and elimination.* PLoS Negl Trop Dis, 2014. **8**(8): p. e3014.
62. Linsuke, S., et al., *High Prevalence of Schistosoma mansoni in Six Health Areas of - Kasansa Health Zone, Democratic Republic of the Congo: Short Report.* Plos Neglected Tropical Diseases, 2014. **8**(12).
63. Sady, H., et al., *Prevalence and associated factors of Schistosomiasis among children in Yemen: implications for an effective control programme.* PLoS Negl Trop Dis, 2013. **7**(8): p. e2377.
64. Barry, M.A., et al., *Global trends in neglected tropical disease control and elimination: impact on child health.* Archives of Disease in Childhood, 2013. **98**(8): p. 635-641.
65. Savioli, L., et al., *Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis.* Parasitology International, 2004. **53**(2): p. 103-113.
66. Savioli, L., et al., *Schistosomiasis and soil-transmitted helminth infections: forging control efforts.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2002. **96**(6): p. 577-579.
67. Mazzitelli, M., et al., *A case report of Schistosoma haematobium infection in a pregnant migrant raises concerns about lack of screening policies.* J Travel Med, 2016. **24**(1).
68. Lengeler, C., et al., *Simple school questionnaires can map both Schistosoma mansoni and Schistosoma haematobium in the Democratic Republic of Congo.* Acta Tropica, 2000. **74**(1): p. 77-87.
69. Lodh, N., et al., *Diagnosis of Schistosoma mansoni without the Stool: Comparison of Three Diagnostic Tests to Detect Schiostosoma mansoni Infection from Filtered Urine in Zambia.* American Journal of Tropical Medicine and Hygiene, 2013. **89**(1): p. 46-50.
70. Utzinger, J., et al., *New diagnostic tools in schistosomiasis.* Clin Microbiol Infect, 2015. **21**(6): p. 529-42.
71. Abdel-Wahab, M.F., et al., *The epidemiology of schistosomiasis in Egypt: Methods, training, and quality control of clinical and ultrasound examinations.* American Journal of Tropical Medicine and Hygiene, 2000. **62**(2): p. 17-20.
72. El-Hawey, A.M., et al., *The epidemiology of schistosomiasis in Egypt: Gharbia Governorate.* Am J Trop Med Hyg, 2000. **62**(2 Suppl): p. 42-8.
73. El-Khoby, T., et al., *The epidemiology of schistosomiasis in Egypt: summary findings in nine governorates.* Am J Trop Med Hyg, 2000. **62**(2 Suppl): p. 88-99.

74. Asztely, M.S., et al., *Is ultrasonography useful for population studies on schistosomiasis mansoni? An evaluation based on a survey on a population from Kome Island, Tanzania.* Acta Radiol Open, 2016. **5**(12): p. 2058460116686392.
75. Stothard, J.R., et al., *Diagnostics for schistosomiasis in Africa and Arabia: a review of present options in control and future needs for elimination.* Parasitology, 2014. **141**(14): p. 1947-61.
76. WHO. *Strategic and business plan for the african network for drugs and diagnostics innovation (ANDI).* 2019; Available from: https://www.who.int/tdr/publications/documents/sbp_andi.pdf?ua=1.
77. Doenhoff, M.J., P.L. Chiodini, and J.V. Hamilton, *Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies?* Trends in Parasitology, 2004. **20**(1): p. 35-39.
78. Enk, M.J., et al., *The effect of the number of stool samples on the observed prevalence and the infection intensity with Schistosoma mansoni among a population in an area of low transmission.* Acta Tropica, 2008. **108**(2-3): p. 222-228.
79. Katz, N., A. Chaves, and J. Pellegrino, *A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni.* Rev Inst Med Trop Sao Paulo, 1972. **14**(6): p. 397-400.
80. Utzinger, J., et al., *FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2008. **102**(1): p. 84-90.
81. Schnyder, M., et al., *Comparison of faecal techniques including FLOTAC for copromicroscopic detection of first stage larvae of Angiostrongylus vasorum.* Parasitology Research, 2011. **109**(1): p. 63-69.
82. Knopp, S., et al., *A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2009. **103**(4): p. 347-354.
83. Knopp, S., et al., *Diagnostic Accuracy of Kato-Katz and FLOTAC for Assessing Anthelmintic Drug Efficacy.* Plos Neglected Tropical Diseases, 2011. **5**(4).
84. Cringoli, G., et al., *Fill-it, float-it: FLOTAC and Mini-FLOTAC for helminth diagnosis.* Tropical Medicine & International Health, 2013. **18**: p. 10-10.
85. Cringoli, G., et al., *FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans.* Nature Protocols, 2010. **5**(3): p. 503-515.
86. Pinheiro, M.C.C., et al., *The combination of three faecal parasitological methods to improve the diagnosis of schistosomiasis mansoni in a low endemic setting in the state of Ceara, Brazil.* Memorias Do Instituto Oswaldo Cruz, 2012. **107**(7): p. 873-876.
87. de Souza, R.P., et al., *Criteria for identification of Schistosoma mansoni eggs in faecal sediments prepared with the Helmintex method and stained by ninhydrin.* Memorias Do Instituto Oswaldo Cruz, 2019. **114**.
88. Favero, V., et al., *Optimization of the Helmintex method for schistosomiasis diagnosis.* Experimental Parasitology, 2017. **177**: p. 28-34.
89. Lindholz, C.G., et al., *Study of diagnostic accuracy of Helmintex, Kato-Katz, and POC-CCA methods for diagnosing intestinal schistosomiasis in Candeal, a low intensity transmission area in northeastern Brazil.* Plos Neglected Tropical Diseases, 2018. **12**(3).
90. Oliveira, W.J., et al., *Evaluation of diagnostic methods for the detection of intestinal schistosomiasis in endemic areas with low parasite loads: Saline gradient, Helmintex, Kato-Katz and rapid urine test.* Plos Neglected Tropical Diseases, 2018. **12**(2).
91. Caldeira, K., et al., *Comparison of the Kato-Katz and Helmintex methods for the diagnosis of schistosomiasis in a low-intensity transmission focus in Bandeirantes, Parana, southern Brazil.* Memorias Do Instituto Oswaldo Cruz, 2012. **107**(5): p. 690-692.
92. Qian, M.B., et al., *Accuracy of the Kato-Katz method and formalin-ether concentration technique for the diagnosis of Clonorchis sinensis, and implication for assessing drug efficacy.* Parasites & Vectors, 2013. **6**.
93. Pontes, L.A., et al., *Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with Schistosoma mansoni.* American Journal of Tropical Medicine and Hygiene, 2003. **68**(6): p. 652-656.

94. Shane, H.L., et al., *Evaluation of Urine CCA Assays for Detection of Schistosoma mansoni Infection in Western Kenya.* Plos Neglected Tropical Diseases, 2011. **5**(1).
95. Stothard, J.R., et al., *Schistosoma mansoni Infections in Young Children: When Are Schistosome Antigens in Urine, Eggs in Stool and Antibodies to Eggs First Detectable?* Plos Neglected Tropical Diseases, 2011. **5**(1).
96. Bergquist, N.R., *Present Aspects of Immunodiagnosis of Schistosomiasis.* Memorias Do Instituto Oswaldo Cruz, 1992. **87**: p. 29-38.
97. Kinkel, H.F., et al., *Evaluation of Eight Serological Tests for Diagnosis of Imported Schistosomiasis.* Clinical and Vaccine Immunology, 2012. **19**(6): p. 948-953.
98. Coulibaly, J.T., et al., *Accuracy of Urine Circulating Cathodic Antigen (CCA) Test for Schistosoma mansoni Diagnosis in Different Settings of Cote d'Ivoire.* Plos Neglected Tropical Diseases, 2011. **5**(11).
99. Coulibaly, J.T., et al., *Accuracy of Urine Circulating Cathodic Antigen Test for the Diagnosis of Schistosoma mansoni in Preschool-Aged Children before and after Treatment.* Plos Neglected Tropical Diseases, 2013. **7**(3).
100. Mutapi, F., et al., *Differential recognition patterns of Schistosoma haematobium adult worm antigens by the human antibodies IgA, IgE, IgG1 and IgG4.* Parasite Immunology, 2011. **33**(3): p. 181-192.
101. de Noya, B.A., et al., *Detection of schistosomiasis cases in low-transmission areas based on coprologic and serologic criteria - The Venezuelan experience.* Acta Tropica, 2007. **103**(1): p. 41-49.
102. Ferrari, T.C.A., *A laboratory test for the diagnosis of neuroschistosomiasis.* Neurological Research, 2010. **32**(3): p. 252-262.
103. Cnops, L., et al., *Schistosoma real-time PCR as diagnostic tool for international travellers and migrants.* Tropical Medicine & International Health, 2012. **17**(10): p. 1208-1216.
104. Mafe, M.A., et al., *Control of urinary schistosomiasis: an investigation into the effective use of questionnaires to identify high-risk communities and individuals in Niger State, Nigeria.* Tropical Medicine & International Health, 2000. **5**(1): p. 53-63.
105. Sheele, J.M., et al., *Evaluation of a novel rapid diagnostic test for Schistosoma haematobium based on the detection of human immunoglobulins bound to filtered Schistosoma haematobium eggs.* Tropical Medicine & International Health, 2013. **18**(4): p. 477-484.
106. Morenikeji, O., et al., *A cross-sectional study on urogenital schistosomiasis in children; haematuria and proteinuria as diagnostic indicators in an endemic rural area of Nigeria.* African Health Sciences, 2014. **14**(2): p. 390-396.
107. Cavalcanti, M.G., et al., *Schistosomiasis in areas of low endemicity: a new era in diagnosis.* Trends in Parasitology, 2013. **29**(2): p. 75-82.
108. Mharakurwa, S., et al., *PCR detection of Plasmodium falciparum in human urine and saliva samples.* Malaria Journal, 2006. **5**.
109. Reithinger, R. and J.C. Dujardin, *Molecular diagnosis of leishmaniasis: Current status and future applications.* Journal of Clinical Microbiology, 2007. **45**(1): p. 21-25.
110. Pontes, L.A., E. Dias-Neto, and A. Rabello, *Detection by polymerase chain reaction of Schistosoma mansoni DNA in human serum and feces.* American Journal of Tropical Medicine and Hygiene, 2002. **66**(2): p. 157-162.
111. Gomes, L.I., M.J. Enk, and A. Rabello, *Diagnosing schistosomiasis: where are we?* Revista Da Sociedade Brasileira De Medicina Tropical, 2014. **47**(1): p. 3-11.
112. Enk, M.J., G.O.E. Silva, and N.B. Rodrigues, *Diagnostic Accuracy and Applicability of a PCR System for the Detection of Schistosoma mansoni DNA in Human Urine Samples from an Endemic Area.* Plos One, 2012. **7**(6).
113. Tomiotto-Pellissier, F., et al., *Nanotechnology as a potential therapeutic alternative for schistosomiasis.* Acta Tropica, 2017. **174**: p. 64-71.
114. Borrego-Sanchez, A., et al., *Molecular and crystal structure of praziquantel. Spectroscopic properties and crystal polymorphism.* European Journal of Pharmaceutical Sciences, 2016. **92**: p. 266-275.
115. Frezza, T.F., et al., *Liposomal-praziquantel: Efficacy against Schistosoma mansoni in a preclinical assay.* Acta Tropica, 2013. **128**(1): p. 70-75.

116. El-Moslemany, R.M., et al., *Miltefosine lipid nanocapsules: Intersection of drug repurposing and nanotechnology for single dose oral treatment of pre-patent schistosomiasis mansoni.* Acta Tropica, 2016. **159**: p. 142-148.
117. Xia, C.C., et al., *Village-based spatio-temporal cluster analysis of the schistosomiasis risk in the Poyang Lake Region, China.* Parasites & Vectors, 2017. **10**.
118. WHO, *Les bilharzioses humaines au Congo Belge et au Ruanda-Urundi.* 1954.
119. Hoover, C.M., et al., *Modelled effects of prawn aquaculture on poverty alleviation and schistosomiasis control.* Nature Sustainability, 2019. **2**(7): p. 611-620.
120. Merrifield, M., et al., *Advancing a vaccine to prevent human schistosomiasis.* Vaccine, 2016. **34**(26): p. 2988-2991.
121. Sotillo, J., et al., *Extracellular vesicles secreted by Schistosoma mansoni contain protein vaccine candidates.* International Journal for Parasitology, 2016. **46**(1): p. 1-5.
122. WHO, *Atlas of the global distribution of schistosomiais* 1987.
123. Madinga, J., et al., *Schistosomiasis in the Democratic Republic of Congo: a literature review.* Parasites & Vectors, 2015. **8**.
124. Bennike, T., F. Frandsen, and G. Mandahlbarth, *Schistosomiasis in Kinshasa - Autochthonous Disease.* Scandinavian Journal of Infectious Diseases, 1976. **8**(3): p. 223-223.
125. Colaert, J., et al., *Presence of a Small Locus of Schistosoma-Mansoni Bilharziasis in Kinshasa, Zaire-Republic.* Annales De La Societe Belge De Medecine Tropicale, 1977. **57**(3): p. 157-162.
126. da Luz, R.I., et al., *Assessment of schistosomiasis and soil-transmitted helminths prevalence in school-aged children and opportunities for integration of control in local health services in Kwilu Province, the Democratic Republic of the Congo.* Tropical Medicine & International Health, 2017. **22**(11): p. 1442-1450.
127. da Luz, R.I., et al., *Schistosomiasis and soil-transmitted helminths prevalence in school-aged children and opportunities for integration of control in health services in Kwilu province, Democratic Republic of the Congo.* Tropical Medicine & International Health, 2017. **22**: p. 243-244.
128. Tchuente, L.A.T., et al., *Epidemiological and genetic observations on human schistosomiasis in Kinshasa, Zaire.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1997. **91**(3): p. 263-269.
129. Hotez, P.J., *The neglected tropical diseases and their devastating health and economic impact on the member nations of the Organisation of the Islamic Conference.* PLoS Negl Trop Dis, 2009. **3**(10): p. e539.
130. Lo, N.C., et al., *A call to strengthen the global strategy against schistosomiasis and soil-transmitted helminthiasis: the time is now.* Lancet Infectious Diseases, 2017. **17**(2): p. E64-E69.
131. Muller, G., J.K. Murenzi, and R. Tulu, *Prevalence of Intestinal Schistosomiasis in Zairian and Ugandan Children in the Aru Zone of Upper Zaire.* Annales De La Societe Belge De Medecine Tropicale, 1986. **66**(3): p. 225-233.
132. ENVISION, *Democratic Republic of the Congo Work Plan.* 2018.
133. Kabore, A., et al., *Initiating NTD programs targeting schistosomiasis and soil-transmitted helminthiasis in two provinces of the Democratic Republic of the Congo: Establishment of baseline prevalence for mass drug administration.* Acta Tropica, 2017. **166**: p. 177-185.
134. Ministry of Health, D., *Plan stratégique de lutte contre les maladies tropicales négligées à chimiothérapie préventive 2016-2020.* 2016.
135. King, C.H., et al., *A 5-year progress report from SCORE, the Schistosomiasis Consortium for Operational Research and Evaluation.* Tropical Medicine & International Health, 2015. **20**: p. 53-53.
136. Steinmann, P., et al., *Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk.* Lancet Infectious Diseases, 2006. **6**(7): p. 411-425.
137. Assare, R.K., et al., *The spatial distribution of Schistosoma mansoni infection in four regions of western Cote d'Ivoire.* Geospatial Health, 2015. **10**(1): p. 69-79.
138. Gordon, C.A., et al., *Asian Schistosomiasis: Current Status and Prospects for Control Leading to Elimination.* Trop Med Infect Dis, 2019. **4**(1).

139. Sun, L.P., et al., *Approaches being used in the national schistosomiasis elimination programme in China: a review*. Infectious Diseases of Poverty, 2017. **6**.
140. Sun, L.P., et al., *A multidisciplinary, integrated approach for the elimination of schistosomiasis: a longitudinal study in a historically hyperendemic region in the lower reaches of the Yangtze River, China from 2005 to 2014.* Infectious Diseases of Poverty, 2017. **6**.
141. WHO, *Preventive chemotherapy in human helminthiasis.* 2006.
142. Montresor, A., *Arithmetic or geometric means of eggs per gram are not appropriate indicators to estimate the impact of control measures in helminth infections.* Trans R Soc Trop Med Hyg, 2007. **101**(8): p. 773-6.
143. WHO, *Prevention and control of schistosomiasis and soil-transmitted helminthiasis.* 2002.
144. Diagnostics, R.M., *Rapid diagnostic tests (RDT) for qualitative detection of Bilharzia (Schistosoma). Pamphlet.* 2015.
145. Coelho, P.M.Z., et al., *Improvement of POC-CCA Interpretation by Using Lyophilization of Urine from Patients with Schistosoma mansoni Low Worm Burden: Towards an Elimination of Doubts about the Concept of Trace.* Plos Neglected Tropical Diseases, 2016. **10**(6).
146. Fernandez-Soto, P., et al., *A Loop-Mediated Isothermal Amplification (LAMP) Assay for Early Detection of Schistosoma mansoni in Stool Samples: A Diagnostic Approach in a Murine Model*. Plos Neglected Tropical Diseases, 2014. **8**(9).
147. Gao, F.H., et al., *Fine scale Spatial-temporal cluster analysis for the infection risk of Schistosomiasis japonica using space-time scan statistics.* Parasites & Vectors, 2014. **7**.
148. Kloos, H., et al., *Combined methods for the study of water contact behavior in a rural schistosomiasis-endemic area in Brazil.* Acta Tropica, 2006. **97**(1): p. 31-41.
149. Taman, A. and S.N. El-Beshbishi, *Laboratory diagnosis of schistosomiasis mansoni: Current status and future trends.* Asian Pacific Journal of Tropical Medicine, 2019. **12**(6): p. 243-249.
150. Lu, Y., et al., *Identification and profiling of circulating antigens by screening with the sera from schistosomiasis japonica patients.* Parasites & Vectors, 2012. **5**.
151. Gaze, S., et al., *An Immunomics Approach to Schistosome Antigen Discovery: Antibody Signatures of Naturally Resistant and Chronically Infected Individuals from Endemic Areas.* Plos Pathogens, 2014. **10**(3).
152. Olveda, D.U., et al., *Bilharzia in the Philippines: past, present, and future.* International Journal of Infectious Diseases, 2014. **18**: p. 52-56.
153. Patz, J.A., et al., *Effects of environmental change on emerging parasitic diseases.* International Journal for Parasitology, 2000. **30**(12-13): p. 1395-1405.
154. Adriko, M., et al., *Data on the pre-MDA and post MDA interventions for Schistosoma mansoni and Schistosoma haematobium in a co-endemic focus in Uganda: 1951-2011*. Data in Brief, 2018. **20**: p. 991-998.
155. Ross, A.G.P. and Y.S. Li, *Mass Drug Administration (MDA) for Schistosomiasis Reply.* Journal of Infectious Diseases, 2015. **211**(5): p. 849-+.
156. Ross, A.G.P., et al., *Can Mass Drug Administration Lead to the Sustainable Control of Schistosomiasis?* Journal of Infectious Diseases, 2015. **211**(2): p. 283-289.
157. Ross, A.G.P., R.M. Olveda, and Y.S. Li, *An audacious goal: the elimination of schistosomiasis in our lifetime through mass drug administration.* Lancet, 2015. **385**(9983): p. 2220-2221.
158. Ibironke, O., et al., *Validation of a New Test for Schistosoma haematobium Based on Detection of Dra1 DNA Fragments in Urine: Evaluation through Latent Class Analysis.* Plos Neglected Tropical Diseases, 2012. **6**(1).
159. Carvalho, G.B.F., et al., *Identification of Schistosoma mansoni candidate antigens for diagnosis of schistosomiasis.* Memorias Do Instituto Oswaldo Cruz, 2011. **106**(7): p. 837-843.
160. Zhu, H.Q., et al., *Comparison of the Miracidium Hatching Test and Modified Kato-Katz Method for Detecting Schistosoma Japonicum in Low Prevalence Areas of China.* Southeast Asian Journal of Tropical Medicine and Public Health, 2014. **45**(1): p. 20-25.
161. Coelho, P.M.Z., et al., *Use of a saline gradient for the diagnosis of schistosomiasis.* Memorias Do Instituto Oswaldo Cruz, 2009. **104**(5): p. 720-723.
162. da Frota, S.M., et al., *Combination of Kato-Katz faecal examinations and ELISA to improve accuracy of diagnosis of intestinal schistosomiasis in a low-endemic setting in Brazil.* Acta Tropica, 2011. **120**: p. S138-S141.

163. Jurberg, A.D., et al., *A new miracidia hatching device for diagnosing schistosomiasis.* Memorias Do Instituto Oswaldo Cruz, 2008. **103**(1): p. 112-114.
164. Karl, S., et al., *The Iron Distribution and Magnetic Properties of Schistosome Eggshells: Implications for Improved Diagnostics.* Plos Neglected Tropical Diseases, 2013. **7**(5).
165. WHO, *The control of schistosomiasis : second report of the WHO Expert Committee [meeting held in Geneva from 8-15 November 1991].* 1991.
166. Deelder, A.M., et al., *Sensitive Determination of Circulating Anodic Antigen in Schistosoma-Mansoni Infected Individuals by an Enzyme-Linked Immunosorbent-Assay Using Monoclonal-Antibodies.* American Journal of Tropical Medicine and Hygiene, 1989. **40**(3): p. 268-272.
167. Deelder, A.M., et al., *Quantitative-Determination of Circulating Antigens in Human Schistosomiasis Mansoni Using an Indirect Hemagglutination Assay.* American Journal of Tropical Medicine and Hygiene, 1989. **40**(1): p. 50-54.
168. Deelder, A.M., et al., *Recognition of Gut-Associated Antigens by Immunoglobulin-M in the Indirect Fluorescent-Antibody Test for Schistosomiasis Mansoni.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1989. **83**(3): p. 364-367.
169. Dejonge, N., O.C. Boerman, and A.M. Deelder, *Time-Resolved Immunofluorometric Assay (Tr-Ifma) for the Detection of the Schistosome Circulating Anodic Antigen.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1989. **83**(5): p. 659-663.
170. Dejonge, N., et al., *Circulating Anodic Antigen Levels in Serum before and after Chemotherapy with Praziquantel in Schistosomiasis Mansoni.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1989. **83**(3): p. 368-372.
171. Dejonge, N., et al., *Presence of the Schistosome Circulating Anodic Antigen (Caa) in Urine of Patients with Schistosoma-Mansoni or S-Haematobium Infections.* American Journal of Tropical Medicine and Hygiene, 1989. **41**(5): p. 563-569.
172. Dejonge, N., et al., *Presence of Circulating Anodic Antigen in Serum of Schistosoma-Intercalatum-Infected Patients from Gabon.* Acta Tropica, 1989. **46**(2): p. 115-120.
173. Dejonge, N., *Detection of the Circulating Anodic Antigen for Immunodiagnosis of Schistosoma Infections.* Immunodiagnostic Approaches in Schistosomiasis, 1992: p. 111-124.
174. Gundersen, S.G., et al., *Quantitative Detection of Schistosomal Circulating Anodic Antigen by a Magnetic Bead Antigen Capture Enzyme-Linked-Immunosorbent-Assay (Mbac-Eia) before and after Mass Chemotherapy.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1992. **86**(2): p. 175-178.
175. Shaker, Z.A., et al., *Detection of Circulating Anodic Antigen before and after Specific Chemotherapy in Experimental Murine Schistosomiasis-Mansoni.* International Journal of Immunopharmacology, 1992. **14**(2): p. 151-158.
176. Vanlieshout, L., et al., *Improved Diagnostic Performance of the Circulating Antigen-Assay in Human Schistosomiasis by Parallel Testing for Circulating Andodic and Cathodic Antigens in Serum and Urine.* American Journal of Tropical Medicine and Hygiene, 1992. **47**(4): p. 463-469.
177. Vantwout, A.B., et al., *Schistosome Circulating Anodic Antigen in Serum of Individuals Infected with Schistosoma-Japonicum from the Philippines before and after Chemotherapy with Praziquantel.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1992. **86**(4): p. 410-413.
178. Cesari, I.M., *Immunological Response in Schistosomiasis-Mansoni.* Interciencia, 1990. **15**(2): p. 76-85.
179. Berggren, W.L. and T.H. Weller, *Immunoelectrophoretic Demonstration of Specific Circulating Antigen in Animals Infected with Schistosoma Mansoni.* American Journal of Tropical Medicine and Hygiene, 1967. **16**(5): p. 606-&.
180. Nash, T.E., *Localization of Circulating Antigen within Gut of Schistosoma-Mansoni.* American Journal of Tropical Medicine and Hygiene, 1974. **23**(6): p. 1085-1087.
181. Carlier, Y., D. Bout, and A. Capron, *Further-Studies on the Circulating M Antigen in Human and Experimental Schistosoma-Mansoni Infections.* Annales D Immunologie, 1978. **C129**(6): p. 811-818.

182. Deelder, A.M., et al., *Schistosoma-Mansoni - Characterization of 2 Circulating Polysaccharide Antigens and the Immunological Response to These Antigens in Mouse, Hamster, and Human Infections.* Experimental Parasitology, 1980. **50**(1): p. 16-32.
183. Feldmeier, H., et al., *Detection and Quantification of Circulating Antigen in Schistosomiasis by Monoclonal-Antibody .2. The Quantification of Circulating Antigens in Human Schistosomiasis-Mansoni and Hematobium - Relationship to Intensity of Infection and Disease Status.* Clinical and Experimental Immunology, 1986. **65**(2): p. 232-243.
184. Sarhan, R.M., et al., *Comparative analysis of the diagnostic performance of adult, cercarial and egg antigens assessed by ELISA, in the diagnosis of chronic human Schistosoma mansoni infection.* Parasitology Research, 2014. **113**(9): p. 3467-3476.
185. Zhou, Y.B., H.M. Zheng, and Q.W. Jiang, *A diagnostic challenge for schistosomiasis japonica in China: consequences on praziquantel-based morbidity control.* Parasites & Vectors, 2011. **4**.
186. Colley, D.G., et al., *A Five-Country Evaluation of a Point-of-Care Circulating Cathodic Antigen Urine Assay for the Prevalence of Schistosoma mansoni.* American Journal of Tropical Medicine and Hygiene, 2013. **88**(3): p. 426-432.
187. Bergquist, N.R., *Immunodiagnosis of Schistosomiasis.* Immunodiagnostic Approaches in Schistosomiasis, 1992: p. 1-8.
188. Grenfell, R.F.Q., et al., *Innovative methodology for point-of-care circulating cathodic antigen with rapid urine concentration for use in the field for detecting low Schistosoma mansoni infection and for control of cure with high accuracy.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2018. **112**(1): p. 1-7.
189. Grenfell, R.F.Q., et al., *Newly Established Monoclonal Antibody Diagnostic Assays for Schistosoma mansoni Direct Detection in Areas of Low Endemicity.* Plos One, 2014. **9**(1).
190. Moraes, V., et al., *Antigens with the potential to differentiate between acute and chronic phases of Schistosoma mansoni.* Faseb Journal, 2014. **28**(1).
191. Corstjens, P.L.A.M., et al., *Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum.* Journal of Clinical Microbiology, 2008. **46**(1): p. 171-176.
192. Adriko, M., et al., *Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for Schistosoma mansoni in different transmission settings within Bugiri District, Uganda.* Acta Tropica, 2014. **136**: p. 50-57.
193. Standley, C.J., et al., *Confirmed local endemicity and putative high transmission of Schistosoma mansoni in the Sesse Islands, Lake Victoria, Uganda.* Parasites & Vectors, 2011. **4**.
194. Polman, K., et al., *Epidemiologic Application of Circulating Antigen-Detection in a Recent Schistosoma-Mansoni Focus in Northern Senegal.* American Journal of Tropical Medicine and Hygiene, 1995. **53**(2): p. 152-157.
195. Vanlieshout, L., et al., *Analysis of Worm Burden Variation in Human Schistosoma-Mansoni Infections by Determination of Serum Levels of Circulating Anodic Antigen and Circulating Cathodic Antigen.* Journal of Infectious Diseases, 1995. **172**(5): p. 1336-1342.
196. Vantwout, A.B., et al., *Serum Levels of Circulating Anodic Antigen and Circulating Cathodic Antigen Detected in Mice Infected with Schistosoma-Japonicum or Schistosoma-Mansoni.* Parasitology Research, 1995. **81**(5): p. 434-437.
197. Bergquist, R., *Prospects of Vaccination against Schistosomiasis.* Scandinavian Journal of Infectious Diseases, 1990: p. 60-71.
198. Gentile, R., et al., *Evaluation of immunological, parasitological and molecular methods for the diagnosis of Schistosoma mansoni infection before and after chemotherapy treatment with praziquantel in experimentally infected Nectomys squamipes.* Veterinary Parasitology, 2011. **180**(3-4): p. 243-249.
199. Grenfell, R.F.Q., et al., *Schistosoma mansoni in a low-prevalence area in Brazil: the importance of additional methods for the diagnosis of hard-to-detect individual carriers by low-cost immunological assays.* Memorias Do Instituto Oswaldo Cruz, 2013. **108**(3): p. 328-334.
200. Ross, A.G.P., et al., *Current concepts - Schistosomiasis.* New England Journal of Medicine, 2002. **346**(16): p. 1212-1220.

201. Hamburger, J., et al., *A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (Schistosoma mansoni) from very early prepatency.* American Journal of Tropical Medicine and Hygiene, 1998. **59**(6): p. 872-876.
202. Hamburger, J., et al., *Development and laboratory evaluation of a polymerase chain reaction for monitoring Schistosoma mansoni infestation of water.* American Journal of Tropical Medicine and Hygiene, 1998. **59**(3): p. 468-473.
203. Kjetland, E.F., et al., *Schistosomiasis PCR in Vaginal Lavage as an Indicator of Genital Schistosoma haematobium Infection in Rural Zimbabwean Women.* American Journal of Tropical Medicine and Hygiene, 2009. **81**(6): p. 1050-1055.
204. Lier, T., et al., *Real-time PCR for detection of low intensity Schistosoma japonicum infections in a pig model.* Acta Tropica, 2008. **105**(1): p. 74-80.
205. ten Hove, R.J., et al., *Multiplex real-time PCR for the detection and quantification of Schistosoma mansoni and S-haematobium infection in stool samples collected in northern Senegal.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2008. **102**(2): p. 179-185.
206. Oliveira, L.M.A., et al., *Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity Schistosoma mansoni infection.* Diagnostic Microbiology and Infectious Disease, 2010. **68**(4): p. 416-421.
207. Gomes, L.I., et al., *Development and Evaluation of a Sensitive PCR-ELISA System for Detection of Schistosoma Infection in Feces.* Plos Neglected Tropical Diseases, 2010. **4**(4).
208. Lodh, N., *Diagnosis of Schistosoma mansoni without the Stool: Comparison of Three Diagnostic Tests to Detect Schiostosoma mansoni Infection from Filtered Urine in Zambia (vol 89, pg 46, 2013).* American Journal of Tropical Medicine and Hygiene, 2013. **89**(3): p. 608-608.
209. Wichmann, D., et al., *Prospective European-wide multicentre study on a blood based real-time PCR for the diagnosis of acute schistosomiasis.* Bmc Infectious Diseases, 2013. **13**.
210. Obeng, B.B., et al., *Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana.* Annals of Tropical Medicine and Parasitology, 2008. **102**(7): p. 625-633.
211. Notomi, T., et al., *Loop-mediated isothermal amplification of DNA.* Nucleic Acids Research, 2000. **28**(12).
212. Njiru, Z.K., *Loop-Mediated Isothermal Amplification Technology: Towards Point of Care Diagnostics.* Plos Neglected Tropical Diseases, 2012. **6**(6).
213. Kaneko, H., et al., *Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances.* Journal of Biochemical and Biophysical Methods, 2007. **70**(3): p. 499-501.
214. Hamburger, J., et al., *Evaluation of Loop-Mediated Isothermal Amplification Suitable for Molecular Monitoring of Schistosome-Infected Snails in Field Laboratories.* American Journal of Tropical Medicine and Hygiene, 2013. **88**(2): p. 344-351.
215. Mori, Y. and T. Notomi, *Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases.* Journal of Infection and Chemotherapy, 2009. **15**(2): p. 62-69.
216. Wang, C., et al., *Application of DNA-based diagnostics in detection of schistosomal DNA in early infection and after drug treatment.* Parasites & Vectors, 2011. **4**.
217. Xu, J., et al., *Sensitive and rapid detection of Schistosoma japonicum DNA by loop-mediated isothermal amplification (LAMP).* International Journal for Parasitology, 2010. **40**(3): p. 327-331.
218. WHO, *WHO Expert Committee on the Control of Schistosomiasis (1991 : Geneva, Switzerland) & World Health Organization. (1993). The control of schistosomiasis : second report of the WHO Expert Committee [meeting held in Geneva from 8-15 November 1991]. World Health Organization.* 1991.
219. Dittrich, P.S. and A. Manz, *Lab-on-a-chip: microfluidics in drug discovery.* Nature Reviews Drug Discovery, 2006. **5**(3): p. 210-218.

220. Lee, H.L., et al., *A Multi-Layered Microfluidic Device for Magnetophoretic Cell Separation.* Biodevices 2009: Proceedings of the International Conference on Biomedical Electronics and Devices, 2009: p. 286-289.
221. Lee, S.H., et al., *Effective mixing in a microfluidic chip using magnetic particles.* Lab on a Chip, 2009. **9**(3): p. 479-482.
222. Li, C.Y., et al., *Rapid nanoliter DNA hybridization based on reciprocating flow on a compact disk microfluidic device.* Analytica Chimica Acta, 2009. **640**(1-2): p. 93-99.
223. Song, J., et al., *Molecular Detection of Schistosome Infections with a Disposable Microfluidic Cassette.* PLoS Negl Trop Dis, 2015. **9**(12): p. e0004318.
224. Lee, W.B., et al., *A Magnetic-bead Based Microfluidic System for Automatic C-reactive protein Detection.* Mnhmt2009, Vol 1, 2010: p. 257-260.
225. Martinez, A.W., et al., *Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices.* Analytical Chemistry, 2010. **82**(1): p. 3-10.
226. Lee, D.W. and Y.H. Cho, *High-radix microfluidic multiplexer with pressure valves of different thresholds.* Lab on a Chip, 2009. **9**(12): p. 1681-1686.
227. Song, J.Z., et al., *Molecular Detection of Schistosome Infections with a Disposable Microfluidic Cassette.* Plos Neglected Tropical Diseases, 2015. **9**(12).
228. Xiao, Y., et al., *A Microfiltration Device for Urogenital Schistosomiasis Diagnostics.* Plos One, 2016. **11**(4).
229. Xiao, Y., et al., *Specific capture and release of circulating tumor cells using a multifunctional nanofiber-integrated microfluidic chip.* Nanomedicine (Lond), 2019. **14**(2): p. 183-199.
230. Ishii, S., T. Segawa, and S. Okabe, *Simultaneous Quantification of Multiple Food- and Waterborne Pathogens by Use of Microfluidic Quantitative PCR.* Applied and Environmental Microbiology, 2013. **79**(9): p. 2891-2898.
231. Ribeiro, D.A., et al., *Expression Profiling of Cell Cycle Regulatory Proteins in Oropharyngeal Carcinomas Using Tissue Microarrays.* In Vivo, 2010. **24**(3): p. 315-320.
232. Huyghe, A., P. Francois, and J. Schrenzel, *Characterization of microbial pathogens by DNA microarrays.* Infection Genetics and Evolution, 2009. **9**(5): p. 987-995.
233. Young, N.D., et al., *Whole-genome sequence of Schistosoma haematobium.* Nature Genetics, 2012. **44**(2): p. 221-225.
234. Walker, A.J., *Insights into the functional biology of schistosomes.* Parasites & Vectors, 2011. **4**.
235. Driguez, P., et al., *Schistosomiasis vaccine discovery using immunomics.* Parasites & Vectors, 2010. **3**.
236. McWilliam, H.E.G., et al., *Novel immunomic technologies for schistosome vaccine development.* Parasite Immunology, 2012. **34**(5): p. 276-284.
237. McWilliam, H.E.G., et al., *Discovery of novel Schistosoma japonicum antigens using a targeted protein microarray approach.* Parasites & Vectors, 2014. **7**.
238. Wu, Y.Q., et al., *Diagnosis, Monitoring, and Control of Schistosomiasis-An Update.* Journal of Biomedical Nanotechnology, 2018. **14**(3): p. 430-455.
239. Liu, G.D., et al., *Renewable amperometric immunosensor for Schistosoma japonium antibody assay.* Anal Chem, 2001. **73**(14): p. 3219-26.
240. Liu, D. and W. Jin, *Amperometric detection of perphenazine at a carbon fiber micro-disk bundle electrode by capillary zone electrophoresis.* J Chromatogr B Analyt Technol Biomed Life Sci, 2003. **789**(2): p. 411-5.
241. Weerakoon, K.G., C.A. Gordon, and D.P. McManus, *DNA Diagnostics for Schistosomiasis Control.* Trop Med Infect Dis, 2018. **3**(3).
242. Koukounari, A., et al., *Sensitivities and Specificities of Diagnostic Tests and Infection Prevalence of Schistosoma haematobium Estimated from Data on Adults in Villages Northwest of Accra, Ghana.* American Journal of Tropical Medicine and Hygiene, 2009. **80**(3): p. 435-441.
243. Ajibola, O., et al., *Tools for Detection of Schistosomiasis in Resource Limited Settings.* Med Sci (Basel), 2018. **6**(2).
244. Mabey, D., et al., *Diagnostics for the developing world.* Nature Reviews Microbiology, 2004. **2**(3): p. 231-240.

245. Friedrich, F., Morozov, O. and Hunziker, P., *A compute model for generating high performance computing SoCs on hybrid systems with FPGAs. In FSP 2016; Third International Workshop on FPGAs for Software Programmers (pp. 1-12). VDE.* 2016.
246. Stothard, J.R., et al., *Towards malaria microscopy at the point-of-contact: an assessment of the diagnostic performance of the Newton Nm1 microscope in Uganda.* Parasitology, 2014. **141**(14): p. 1819-25.
247. Hayashida, K., et al., *Direct blood dry LAMP: a rapid, stable, and easy diagnostic tool for Human African Trypanosomiasis.* PLoS Negl Trop Dis, 2015. **9**(3): p. e0003578.
248. Coghlan, B., et al., *Mortality in the Democratic Republic of Congo: a nationwide survey.* Lancet, 2006. **367**(9504): p. 44-51.
249. Naidoo, S. and Institute for Global Dialogue., *The war economy in the Democratic Republic of Congo*. IGD occasional paper. 2003, Braamfontein, South Africa: Institute for Global Dialogue. 91 p.
250. Human Rights Watch (Organization), *The curse of gold : Democratic Republic of Congo*. 2005, New York, NY: Human Rights Watch. 159 p.
251. Baregu, M.L., Southern Africa Regional Institute for Policy Studies., and SAPES Trust., *The crisis in the Democratic Republic of Congo*. SARIPS series. 1999, Mount Pleasant, Harare, Zimbabwe: SAPES Books. v, 224 p.
252. Soderlund, W.C., et al., *Africa's deadliest conflict : media coverage of the humanitarian disaster in the Congo and the United Nations response, 1997-2008*. 2012, Waterloo, Ontario: Wilfrid Laurier University Press. xix, 237 pages.
253. Reyntjens, F., *The great African war : Congo and regional geopolitics, 1996-2006*. 2009, Cambridge ; New York: Cambridge University Press. xi, 327 p.
254. Benedi Sudi Am Mba, M., *How to build a lasting peace in the DRC : make 5 million history*. 2008, Bloomington, IN: AuthorHouse. xvi, 115 p.
255. SENTRY, T., *Or sale et blanchiment d'argent. Commerce aux Etats-Unis et en Europe d'or provenant de zones de conflits dans l'est de la RDC. Résumé.* 2018.
256. Congo (Democratic Republic). Ministère des droits humains., *Livre blanc spécial : sur les récents massacres, actes d'anthropophagie et autres graves violations des droits de l'homme et du Droit international humanitaire commis essentiellement en Ituri, au Nord-Kivu et au Sud-Kivu : (du 1er septembre 2002 au 31 janvier 2003)*. 2003, Kinshasa: République démocratique du Congo, Ministère des droits humains. 133 p.
257. Morris, G. and J.R. Giersdorf, *Choreographies of 21st century wars*. Oxford studies in dance theory. 2016, New York, NY: Oxford University Press. ix, 369 pages.
258. WHO, *Ebola in the Democratic Republic of the Congo. Health Emergency Update.* 2019.
259. Vlassenroot, K., S.v. Bockstael, and Institut royal des relations internationales (Brussels Belgium), *Artisanal diamond mining : perspectives and challenges*. 2008, Gent: Academia Press for EGMONT. xii, 289 p.
260. Abegunrin, O., *Africa in the new world order : peace and security challenges in the twenty-first century*. 2014, Lanham: Lexington Books. pages cm.
261. Richter, I., S. Berking, and R. Müller-Schmid, *Building a transnational civil society : global issues and global actors*. 2006, Basingstoke England ; New York: Palgrave Macmillan. xii, 268 p.
262. Bergquist, R., et al., *Elimination of schistosomiasis: the tools required.* Infectious Diseases of Poverty, 2017. **6**.
263. Colley, D.G., T.S. Andros, and C.H. Campbell, *Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs?* Infectious Diseases of Poverty, 2017. **6**.
264. FAO, *FAO, GIEWS, Earth Observation.* 2019.
265. Ministry of Health, D., *Plan national de développement sanitaire 2016-2020: vers la couverture sanitaire universelle.* 2016.
266. Raeymaekers, C.V.a.T., *Terr(it)or(ies) of peace? The Congolese mining frontier and the fight against "Conflict minerals".* 2016.
267. Raeymaekers, T., *Violent capitalism and hybrid identity in the Eastern Congo : power to the Margins*. 2014, New York, NY: Cambridge University Press. xi, 191 pages.

268. IPIS, *Mapping artisanal areas and mineral supply chains in eastern DR Congo. Impact of armed interference and responsible sourcing.* 2019.
269. IPIS, *Mineral supply chains and conflict links in eastern DRC: 5 years on. DRAFT.* 2015.
270. BARRICK, *Kibali achieves record year and remains committed to partnership philosophy. Presentation.* 2019.
271. Bank, W., *Democratic Republic of Congo systematic country diagnostic. Policy priorities for poverty reduction and shared prosperity in a post-conflict country and fragile state.* 2018.
272. Bank, W., *DRC Economic Report.* 2010.
273. WIKIPEDIA, *First Congo War.* 2019.
274. WIKIPEDIA, *Zweiter Kongokrieg 2001 bis 2003.* 2019.
275. Alert, I., *La complexité de la gestion des ressources dans un contexte de fragilité de l'état: le cas de l'est de la RDC.* 2010.
276. Morozov, O.V., M. Unser, and P. Hunziker, *Reconstruction of large, irregularly sampled multidimensional images. A tensor-based approach.* IEEE Trans Med Imaging, 2011. **30**(2): p. 366-74.
277. McManus, D.P., et al., *Conquering Schistosomiasis in China: The Final Chapter.* American Journal of Tropical Medicine and Hygiene, 2015. **93**(4): p. 558-558.
278. WHO, *Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2016. Weekly epidemiological record. 8 December 2017.* 2017.
279. WHO, *Schistosomiasis country indicators, DR Congo.* 2017.
280. King, C.H., *Parasites and poverty: The case of schistosomiasis.* Acta Tropica, 2010. **113**(2): p. 95-104.
281. WHO, *The social context of schistosomiasis and its control.* 2008.
282. Campbell, S.J., et al., *Tailoring Water, Sanitation, and Hygiene (WASH) Targets for Soil-Transmitted Helminthiasis and Schistosomiasis Control.* Trends in Parasitology, 2018. **34**(1): p. 53-63.
283. Gates, S. and S. Reich, *Child soldiers in the age of fractured states*. The security continuum. 2010, Pittsburgh, Pa.: University of Pittsburgh Press. x, 310 p.
284. UNICEF, *Water, sanitation and hygiene overview.* 2017.
285. Elbaz, T. and G. Esmat, *Hepatic and intestinal schistosomiasis: review.* J Adv Res, 2013. **4**(5): p. 445-52.
286. WHO, *Schistosomiasis: population requiring preventive chemotherapy and number of people treated in 2010.* 2012. **4**(87): p. 37-44.
287. World Health Organization. and D.W.T. Crompton, *Preventive chemotherapy in human helminthiasis : coordinated use of anthelminthic drugs in control interventions : a manual for health professionals and programme managers*. 2006, Geneva, Swizterland: World Health Organization. ix, 62 pages.
288. WHO, *Schistosomiasis Key facts.* 2020.
289. Adenowo, A.F., et al., *Impact of human schistosomiasis in sub-Saharan Africa.* Brazilian Journal of Infectious Diseases, 2015. **19**(2): p. 196-205.
290. WHO, *Schistosomiasis. Key facts.* 2018.
291. Aerssens, C., et al., *Schistosomiasis in Belgian military personnel returning from the Democratic Republic of Congo.* Mil Med, 2011. **176**(11): p. 1341-6.
292. De Clercq, D., *[The malacological situation in Kinshasa and description of an aotochthonous focus of schistosomiasis due to Schistosoma intercalatum].* Ann Soc Belg Med Trop, 1987. **67**(4): p. 345-52.
293. Gryseels, B. and N.P. Ngimbi, *Further observations on the urban Schistosoma mansoni focus in Kinshasa, Zaire.* Ann Soc Belg Med Trop, 1983. **63**(4): p. 341-6.
294. Madinga, J., et al., *Epidemiology of polyparasitism with Taenia solium, schistosomes and soil-transmitted helminths in the co-endemic village of Malanga, Democratic Republic of Congo.* Acta Trop, 2017. **171**: p. 186-193.
295. De Clercq, D., et al., *[Assessment of a focus of schistosomiasis caused by Schistosoma mansoni in Mayombe, Republic of Zaire].* Ann Soc Belg Med Trop, 1985. **65**(2): p. 153-62.

296. Kabore, A., et al., *Initiating NTD programs targeting schistosomiasis and soil-transmitted helminthiasis in two provinces of the Democratic Republic of the Congo: Establishment of baseline prevalence for mass drug administration.* Acta Trop, 2017. **166**: p. 177-185.
297. Polderman, A.M., *Cost-effectiveness of different ways of controlling intestinal schistosomiasis: a case study.* Soc Sci Med, 1984. **19**(10): p. 1073-80.
298. Polderman, A.M., *Schistosomiasis in a mining area: intersectoral implications.* Trop Med Parasitol, 1986. **37**(2): p. 195-9.
299. Polderman, A.M., B. Gryseels, and P. De Caluwe, *Cure rates and egg reduction in treatment of intestinal schistosomiasis with oxamniquine and praziquantel in Maniema, Zaire.* Trans R Soc Trop Med Hyg, 1988. **82**(1): p. 115-6.
300. Polderman, A.M., et al., *Methodology and interpretation of parasitological surveillance of intestinal schistosomiasis in Maniema, Kivu Province, Zaire.* Ann Soc Belg Med Trop, 1985. **65**(3): p. 243-9.
301. Polderman, A.M., et al., *On the distribution and control of schistosomiasis mansoni in Maniema, Zaire.* Acta Leiden, 1982. **49**: p. 17-29.
302. Polderman, A.M., et al., *Historical, geological and ecological aspects of transmission of intestinal schistosomiasis in Maniema, Kivu Province, Zaire.* Ann Soc Belg Med Trop, 1985. **65**(3): p. 251-61.
303. Baluku, B., M. Bagalwa, and B. Bisimwa, *[Parasitologic survey of schistosomiasis due to Schistosoma mansoni in Katana, Democratic Republic of Congo].* Med Trop (Mars), 2000. **60**(2): p. 163-6.
304. Raccurt, C., C. Ripert, and J.A. Grimaud, *[Epidemiological study of intestinal and urinary bilharziasis in the region of the artificial lake of Lufira. 5. Effects of helminthiasis on the health of subjects excreting eggs of Schistosoma mansoni].* Bull Soc Pathol Exot Filiales, 1974. **67**(4): p. 402-22.
305. Monseur, J., et al., *[Epidemiological study of intestinal and urinary bilharziasis in the area of the reservoir lake of the Lufira. 4. Effects of helminthiasis, as evaluated by examination of urological lesions, on the health of subjects passing Schistosoma haematobium eggs].* Bull Soc Pathol Exot Filiales, 1972. **65**(6): p. 822-40.
306. Knoblauch, A.M., et al., *Schistosomiasis and soil-transmitted helminth infections in schoolchildren in north-eastern Democratic Republic of the Congo.* Trans R Soc Trop Med Hyg, 2016. **110**(7): p. 424-6.
307. CAID CD. Cellules d'Analyse des Indicateurs de Développement. Congo, R., *Indicateurs de développement. Données administrative par province. Province de l'Ituri.* 2017.
308. START CENTER. Strategic analysis, r.a.t.c., *DRC survey: an overview of demographics, health, and financial services in the DRC.* . 2017.
309. Program., W.W.a.S., *Water supply and sanitation in the Democratic Republic of Congo. Turning finance into services for 2015 and beyond.* 2011.
310. UNICEF/WHO, *Drinking water, sanitation, and hygiene database.* . 2017.
311. Exum, N.G., et al., *The prevalence of schistosomiasis in Uganda: A nationally representative population estimate to inform control programs and water and sanitation interventions.* Plos Neglected Tropical Diseases, 2019. **13**(8).
312. Doumenge, J.P. and K.E. Mott, *Global distribution of schistosomiasis: CEGET/WHO atlas.* World Health Stat Q, 1984. **37**(2): p. 186-99.
313. Stanton, M.C., et al., *Intestinal schistosomiasis in Uganda at high altitude (>1400 m): malacological and epidemiological surveys on Mount Elgon and in Fort Portal crater lakes reveal extra preventive chemotherapy needs.* Infect Dis Poverty, 2017. **6**(1): p. 34.
314. Walz, Y., et al., *Modeling and Validation of Environmental Suitability for Schistosomiasis Transmission Using Remote Sensing.* PLoS Negl Trop Dis, 2015. **9**(11): p. e0004217.
315. Mandahl-Barth, G., *The species of the genus Bulinus, intermediate hosts of Schistosoma.* Bull World Health Organ, 1965. **33**(1): p. 33-44.
316. Stanton, M.C., et al., *Intestinal schistosomiasis in Uganda at high altitude (> 1400 m): malacological and epidemiological surveys on Mount Elgon and in Fort Portal crater lakes reveal extra preventive chemotherapy needs.* Infectious Diseases of Poverty, 2017. **6**.

317. Bergquist, R., et al., *Elimination of schistosomiasis: the tools required.* Infect Dis Poverty, 2017. **6**(1): p. 158.
318. Bergquist, R., J. Utzinger, and J. Keiser, *Controlling schistosomiasis with praziquantel: How much longer without a viable alternative?* Infect Dis Poverty, 2017. **6**(1): p. 74.
319. Gray, D.J., et al., *Schistosomiasis elimination: lessons from the past guide the future.* Lancet Infect Dis, 2010. **10**(10): p. 733-6.
320. Bakuza, J.S., et al., *Estimating the prevalence and intensity of Schistosoma mansoni infection among rural communities in Western Tanzania: The influence of sampling strategy and statistical approach.* Plos Neglected Tropical Diseases, 2017. **11**(9).
321. Dawaki, S., et al., *Prevalence and Risk Factors of Schistosomiasis among Hausa Communities in Kano State, Nigeria.* Rev Inst Med Trop Sao Paulo, 2016. **58**: p. 54.
322. Gazzinelli, A., et al., *Socioeconomic determinants of schistosomiasis in a poor rural area in Brazil.* Acta Trop, 2006. **99**(2-3): p. 260-71.
323. Gazzinelli, M.F., et al., *The impact of two education methods on knowledge of schistosomiasis transmission and prevention among schoolchildren in a rural community in northern Minas Gerais, Brazil.* Mem Inst Oswaldo Cruz, 2006. **101 Suppl 1**: p. 45-53.
324. Salawu, O.T. and A.B. Odaibo, *Schistosomiasis among pregnant women in rural communities in Nigeria.* Int J Gynaecol Obstet, 2013. **122**(1): p. 1-4.
325. Dabo, A., et al., *[Epidemiology of schistosomiasis in a suburban school area of Bamako (Republic of Mali)].* Bull Soc Pathol Exot, 1995. **88**(1): p. 29-34.
326. Firmo, J.O., et al., *Urban schistosomiasis: morbidity, sociodemographic characteristics and water contact patterns predictive of infection.* Int J Epidemiol, 1996. **25**(6): p. 1292-300.
327. Gryseels, B., L. Nkulikyinka, and D. Engels, *Impact of repeated community-based selective chemotherapy on morbidity due to schistosomiasis mansoni.* Am J Trop Med Hyg, 1994. **51**(5): p. 634-41.
328. Jorgensen, A., T.K. Kristensen, and J.R. Stothard, *Phylogeny and biogeography of African Biomphalaria (Gastropoda : Planorbidae), with emphasis on endemic species of the great East African lakes.* Zoological Journal of the Linnean Society, 2007. **151**(2): p. 337-349.
329. Chipeta, M.G., B. Ngwira, and L.N. Kazembe, *Analysis of Schistosomiasis haematobium Infection Prevalence and Intensity in Chikhwawa, Malawi: An Application of a Two Part Model.* Plos Neglected Tropical Diseases, 2013. **7**(3).
330. Aemero, M., N. Berhe, and B. Erko, *Status of Schistosoma mansoni prevalence and intensity of infection in geographically apart endemic localities of Ethiopia: a comparison.* Ethiopian journal of health sciences, 2014. **24**(3): p. 189-94.
331. Okoyo, C., et al., *Comparing the performance of circulating cathodic antigen and Kato-Katz techniques in evaluating Schistosoma mansoni infection in areas with low prevalence in selected counties of Kenya: a cross-sectional study.* Bmc Public Health, 2018. **18**.
332. Standley, C.J., et al., *Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria.* Parasites & vectors, 2010. **3**(1): p. 7.
333. Nalugwa, A., et al., *Intestinal schistosomiasis among preschool children along the shores of Lake Victoria in Uganda.* Acta tropica, 2015. **142**: p. 115-21.
334. van der Werf, M.J., et al., *Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa.* Acta Trop, 2003. **86**(2-3): p. 125-39.
335. Gryseels, B. and A.M. Polderman, *The morbidity of schistosomiasis mansoni in Maniema (Zaire).* Trans R Soc Trop Med Hyg, 1987. **81**(2): p. 202-9.
336. Ongom, V.L., et al., *Epidemiology and Consequences of Schistosoma-Mansoni Infection in West Nile, Uganda .2. Hospital Investigation of a Sample from Panyagoro Community.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1972. **66**(6): p. 852-863.
337. WHO, *ULTRASOUND IN SCHISTOSOMIASIS. A Practical Guide to the Standarized Use of Ultrasonography for the Assessment of Schistosomiasis-related Morbidity.* World Health Library, 2000.
338. Berhe, N., et al., *Large scale evaluation of WHO's ultrasonographic staging system of schistosomal periportal fibrosis in Ethiopia.* Trop Med Int Health, 2006. **11**(8): p. 1286-94.
339. WHO, *WHO's roadmap 2012-2030.* 2012.

340. WHO, *WHO's Schistosomiasis: progress report 2001 - 2011, strategic plan 2012 – 2020.* 2013.
341. Kaatano, G.M., et al., *Schistosoma mansoni-Related Hepatosplenic Morbidity in Adult Population on Kome Island, Sengerema District, Tanzania.* The Korean journal of parasitology, 2015. **53**(5): p. 545-51.
342. Booth, M., et al., *Hepatosplenic morbidity in two neighbouring communities in Uganda with high levels of Schistosoma mansoni infection but very different durations of residence.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2004. **98**(2): p. 125-36.
343. Maurice Mutro Nigo, G.B.S.-B., Manuel Battegay, Peter Odermatt, Patrick Hunziker., *Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology.* Prec. Nanomed., 2020. **3**(1): p. 439-458.
344. Mazigo, H.D. and J. Heukelbach, *Diagnostic Performance of Kato Katz Technique and Point-of-Care Circulating Cathodic Antigen Rapid Test in Diagnosing Schistosoma mansoni Infection in HIV-1 Co-Infected Adults on the Shoreline of Lake Victoria, Tanzania.* Tropical medicine and infectious disease, 2018. **3**(2).
345. Kinung'hi, S.M., et al., *Coinfection of intestinal schistosomiasis and malaria and association with haemoglobin levels and nutritional status in school children in Mara region, Northwestern Tanzania: a cross-sectional exploratory study.* BMC Res Notes, 2017. **10**(1): p. 583.
346. Mazigo, H.D., et al., *Periportal fibrosis, liver and spleen sizes among S. mansoni mono or co-infected individuals with human immunodeficiency virus-1 in fishing villages along Lake Victoria shores, North-Western, Tanzania.* Parasit Vectors, 2015. **8**: p. 260.
347. Mazigo, H.D., et al., *Co-infection of Schistosoma mansoni/hepatitis C virus and their associated factors among adult individuals living in fishing villages, north-western Tanzania.* BMC Infect Dis, 2017. **17**(1): p. 668.
348. Samuels, A.M., et al., *Schistosoma mansoni morbidity among school-aged children: a SCORE project in Kenya.* The American journal of tropical medicine and hygiene, 2012. **87**(5): p. 874-82.
349. Gryseels, B. and A.M. Polderman, *The Morbidity of Schistosomiasis-Mansoni in Maniema (Zaire).* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1987. **81**(2): p. 202-209.
350. Gryseels, B., *Morbidity and Dynamics of Schistosomiasis after Chemotherapy - Implications for Control.* European Journal of Pharmacology, 1990. **183**(3): p. 670-670.
351. Gryseels, B. and L. Nkulikyinka, *The Morbidity of Schistosomiasis-Mansoni in the Highland Focus of Lake Cohoha, Burundi.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1990. **84**(4): p. 542-547.
352. Gryseels, B. and A.M. Polderman, *Schistosoma-Mansoni Morbidity in 3 Central-Africa Foci - Intrapopulation Vs Interpopulation Analysis.* Tropical Medicine and Parasitology, 1987. **38**(3): p. 263-263.
353. Cole, T.J., et al., *Establishing a standard definition for child overweight and obesity worldwide: international survey.* BMJ, 2000. **320**(7244): p. 1240-3.
354. Parr, J.B., et al., *Pfhrp2-Deleted Plasmodium falciparum Parasites in the Democratic Republic of the Congo: A National Cross-sectional Survey.* The Journal of infectious diseases, 2017. **216**(1): p. 36-44.
355. Makiala-Mandanda, S., et al., *High Prevalence and Diversity of Hepatitis Viruses in Suspected Cases of Yellow Fever in the Democratic Republic of Congo.* Journal of clinical microbiology, 2017. **55**(5): p. 1299-1312.
356. McManus, D.P., et al., *Schistosomiasis.* Nature reviews Disease primers, 2018. **4**(1): p. 13.
357. Adenowo, A.F., et al., *Impact of human schistosomiasis in sub-Saharan Africa.* The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases, 2015. **19**(2): p. 196-205.
358. Gunda, D.W., et al., *Morbidity and Mortality Due to Schistosoma mansoni Related Periportal Fibrosis: Could Early Diagnosis of Varices Improve the Outcome Following Available Treatment Modalities in Sub Saharan Africa? A Scoping Review.* Tropical medicine and infectious disease, 2020. **5**(1).

359. Chofle, A.A., et al., *Oesophageal varices, schistosomiasis, and mortality among patients admitted with haematemesis in Mwanza, Tanzania: a prospective cohort study.* BMC infectious diseases, 2014. **14**: p. 303.
360. Opio, C.K., et al., *The diagnostic accuracy of routine clinical findings for detection of esophageal varices in rural sub-Saharan Africa where schistosomiasis is endemic.* African health sciences, 2019. **19**(4): p. 3225-3234.
361. Moore, C.M. and D.H. Van Thiel, *Cirrhotic ascites review: Pathophysiology, diagnosis and management.* World journal of hepatology, 2013. **5**(5): p. 251-63.
362. Maurice Nigo, M.O., P. Salieb-Beugelaar, GB. Morozov, O. Battegay, M. and Hunziker PR. , *Epidemiology of Schistosomiasis in Ituri Province, Northeastern Democratic Republic of the Congo.* PLoS Negl Trop Dis, Submitted 10 April 2020.
363. Maurice Nigo, M.O., P. Wully, DN. Salieb-Beugelaar, GB. Battegay, M. and Hunziker PR., *Morbidity associated with Schstosoma mansoni in northeastern Democratic Republic of the Congo.* PLoS Negl Trop Dis, Submitted 18 April 2020.
364. RMD, *Schistosomiasis point-of-care circulating cathodic antigen (POC-CCA) cassette based test technical brochure.* Rapid Medical Diagnostics, 2015.
365. WHO, *Haemoglobin measurement: reliability of some simple techniques for use in a primary health care setting.* . Bull World Health Organ, 1983.
366. Aye, P., et al., *Patients with severe schistosomiasis mekongi morbidity demonstrating ongoing transmission in Southern Lao People's Democratic Republic.* Acta tropica, 2020. **204**: p. 105323.
367. Kabwama, S.N., et al., *Risk factors for hematemesis in Hoima and Buliisa Districts, Western Uganda, September-October 2015.* Pan African Medical Journal, 2017. **28**.
368. Garba, A., et al., *Schistosomiasis in infants and preschool-aged children: Infection in a single Schistosoma haematobium and a mixed S. haematobium-S. mansoni foci of Niger.* Acta Trop, 2010. **115**(3): p. 212-9.
369. Dawson, E.M., et al., *Intestinal schistosomiasis in pre school-aged children of Lake Albert, Uganda: diagnostic accuracy of a rapid test for detection of anti-schistosome antibodies.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2013. **107**(10): p. 639-647.

11 Curriculum vitae

Personal information

Full Name	Maurice Mutro Nigo
Place, date of birth	Adi, February 22, 1958
Marital status	Married
Nationality	Congolese (DRC)
Languages	French (very good), English (fair)
Address (work)	Nanomedicine Research Lab CLINAM Bernoullistrasse 20, 4056 Basel, Switzerland
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Education and work experience

02/2015 – 12/2019	PhD in Clinical Science (Epidemiology)
PhD thesis	Schistosomiasis in Eastern Democratic Republic of the Congo: A major neglected healthcare concern
Supervision	Prof Dr Patrick Hunziker (University Hospital Basel), Prof Dr Manuel Battegay (University Hospital Basel), Prof Peter Odermatt (Swiss TPH)
05/2017	Poster, *Schistosomiasis: a widespread crisis in northeastern DR Congo*, CLINAM international conference, Basel, Switerland
09/2018	Presentation: *New data uncovering a major health problem in Eastern Congo: Schistosomiasis*. CLINAM international conference, Basel, Switerland
01/2019	Presentation: *Schistosomiase en Ituri, premiers résultats et implications pour la santé publique*. Journée scientifiques et series de conferences, ISTM-Nyankunde, Bunia, République Démocratique du Congo
06/2017 – 09/2017	In-depth cross-sectional survey of 13 villages in the Ituri province (Principle Investigator)
03/2016 – 09/2016	Large cross-sectional survey of 51 villages in the Ituri pronvince (Principle Investigator)
05/2015 – 09/2015	Exploratory survey of 7 schools in the Ituri pronvince (Principle Investigator)
03/2012 – 10/2014	Chair of the Board of the Regional Centre for Essential Drugs Supply and Distribution in Bunia and Uelé (CADIMEBU), Bunia, DR Congo
10/2008 – 03/2012	Head of laboratory, Phase 3 Moxidectin Trial, Wyeth/Pfzer and WHO/TDR, Rethy centre, DR Congo
03/2006 – 10/2008	Administrative Dean, Institut Supérieur des Techniques Médicales (ISTM), Nyankunde, Bunia, DRC
11/1996 – 03/2006	Teacher in the Institut Supérieur des Techniques Médicales (ISTM) and other universities of Ituri province, DRC

09/1993- 10/1996	Master in Public Health (Epidemiology), Master thesis "*Analyse épidémiologique et caractérisation génotypique d'une épidémie nosocomiale à Pseudomonas pickettii*" Supervised by Prof Dr Patrick De Mol, University of Liège, Belgium
09/1995 – 06/1996	Secondary/Superior Teaching Aggregation Diploma in Public Health, University of Liège, Belgium
10/1984 – 09/1993	Head of laboratory, CME-Nyankunde Hospital, DRC
09/1981 – 10/1984	Bachelor in laboratory techniques, Bachelor essay "*Étude bactériologique des cas de gastro-entérites infantiles aux Cliniques Universitaires de Kinshasa*", Institut Supérieur des Techniques Médicales (ISTM) of Kinshasa, DRC
09/1978 – 09/1981	Nursing work at CME-Nyankunde Hospital, DRC
09/1974 – 06/1978	Gymnasium at Nurse Vocational school, Nyankunde, DRC

Publications

Maurice M Nigo, Peter Odermatt, Georgette B. Salieb-Beugelaar, Manuel Battegay and Patrick Hunziker. Schistosomiasis in Eastern Congo: A major neglected healthcare concern in a setting of war and unrest, extreme population poverty, extreme richness in minerals, minimal infrastructure. View point. *The Lancet*, in preparation

Maurice M Nigo, Peter Odermatt, David Wully Nigo, Georgette B. Salieb-Beugelaar, Manuel Battegay and Patrick Hunziker. Patients with severe intestinal and hepatosplenic Schistosomiasis in the Ituri province, Democratic Republic of the Congo: *Cases Report*. Submitted to: BMC *Infectous Diseases of Poverty*

Maurice M Nigo, Peter Odermatt, David Wully Nigo, Georgette B. Salieb-Beugelaar, Manuel Battegay and Patrick Hunziker. Morbidity associated with *Schistosoma mansoni* in northeastern Democratic Republic of the Congo. Submitted to: *PLoS Neglected Tropical Diseases*

Maurice M Nigo, Peter Odermatt, Georgette B. Salieb-Beugelaar, Oleksii Morozov, Manuel Battegay and Patrick Hunziker. Epidemiology of Schistosomiasis in Ituri province, northeastern Democratic Republic of the Congo. Submitted to: *PLoS Neglected Tropical Diseases*

Maurice M Nigo, Peter Odermatt, Georgette B. Salieb-Beugelaar, Manuel Battegay and Patrick Hunziker. Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology. *Precision Nanomedicine,* 2020, (3) 1: 439-458.

Nicholas O Opoku, Didier K Bakajika, Eric M Kanza, **Maurice M Nigo** et al. Single dose moxidectin versus ivermectin for *Onchocerca volvulus* infection in Ghana, Liberia, and the Democratic Republic of the Congo: a randomised, controlled, double-blind phase 3 trial. *The Lancet*, 2018, 392 (10154): 1207-1216.

Zhang, B., Salieb-Beugelaar, G. B., **Nigo, M. M**., Weidmann, M., & Hunziker, P. Diagnosing dengue virus infection—rapid tests and the role of micro/nanotechnologies. *Nanomedicine: Nanotechnology, Biology and Medicine*, 2015, *11*(7), 1745-1761

Salieb-Beugelaar, G. B., Zhang, B., **Nigo, M. M**., Frischmann, S., & Hunziker, P. R. Improving diagnosis of pneumococcal disease by multiparameter testing and micro/nanotechnologies. *European Journal of Nanomedicine*, 2016, *8*(3), 115-128

Bakajika DK, **Nigo MM**, … Lloyd MM, Weil GJ, Fischer PU et al. Filarial antigenemia and *Loa loa* night blood microfilaremia in an area without bancroftian filariasis in the Democratic Republic of Congo. *The American journal of tropical medicine and hygiene*, 2014, 91(6): 1142-1148

Didier K. Bakajika, **Maurice M. Nigo**, Jean-Pierre Lotsima, Gary J. Weil and Peter U. Fischer. *Polyparasitism Complicates Efforts to Eliminate Lymphatic Filariasis in Central Africa*: Abstract. University School of Medicine, Saint Louis, MO, United States.

Wanji S, Akotshi DO, **Mutro MN**, Tepage F, Ukety TO, Diggle PJ & Remme JH. *Validation of the Rapid Assessment Procedure for Loiasis (RAPLOA) in the Democratic Republic of Congo*. Parasites & Vectors, 2012, 5:25, 1 – 10.

Chetoui H, Melin P, Struelens MJ, Delhalle E, **Mutro Nigo M**, de Ryck R and de Mol P. *Comparison of Biotyping, Ribotyping, and Pulsed-Field Gel Electrophoresis for Investigation of a Common-Source Outbreak of Burkholderia pickettii Bacteremia*. J. Clin. Microbiol. 1997, 35(6): 1398.

12 Appendix

Individual Questionnaire

ENQUÊTE SCHISTOSOMIASE EN ITURI QUESTIONNAIRE INDIVIDUEL								
Données générales								
Date	_____/_____/_____							
Numéro du ménage	|____| |____| |____|							
Numéro d'identification (ID)	|____| |____| |____| |____| |____|							
Village (nom)	_____							
Données démographiques								
Nom et postnom	_____							
Date de naissance :	le _____/ _____/ _____							
Age	_____		Sexe	_____				
Statu matrimonial	Marié	☐	Veuf	☐	Divorcé	☐	Polygame	☐
Nationalité	_____		Tribu	_____				
Données anthropométriques								
Poids	_____ kg (arrondi à 0,5 kg)							
Taille	_____ cm (arrondi à 0,5 cm)							

QUESTIONNAIRE INDIVIDUEL (suite)				
Données socio-économiques				
Occupation (cochez une ou plusieurs cases)				
Aucune	☐			
Ménagère	Oui	☐	Non	☐
Cultivateur	Oui	☐	Non	☐
Pêcheur	Oui	☐	Non	☐
Commercant	Oui	☐	Non	☐
Enseignant(e)	Oui	☐	Non	☐
Fonctionnaire	Oui	☐	Non	☐
Infirmier(ère)	Oui	☐	Non	☐
Médecin	Oui	☐	Non	☐
Avocat	Oui	☐	Non	☐
Pasteur	Oui	☐	Non	☐
Autre (à préciser)	_____			
Education				
Primaire	Complète	☐	Incomplète	☐
Secondaire	Complète	☐	Incomplète	☐
Supérieur	Complète	☐	Incomplète	☐
Universitaire	Complète	☐	Incomplète	☐
Post-universitaire	Complète	☐	Incomplète	☐
Religion				
Animiste	Oui	☐	Non	☐
Catholique	Oui	☐	Non	☐
Protestante	Oui	☐	Non	☐
Reveil	Oui	☐	Non	☐
Kimbanguiste	Oui	☐	Non	☐
Musulman	Oui	☐	Non	☐
Autre (à préciser)	_____			

QUESTIONNAIRE INDIVIDUEL (suite)						
Facteurs de risque : Pratiques (1) – Eau potable						
Quelle eau buvez-vous ? (cochez une ou plusieurs cases)						
L'eau du robinet de la maison	Oui	☐	Non	☐	De temps à temps	☐
L'eau de la source aménagée du village	Oui	☐	Non	☐	De temps à temps	☐
L'eau embouteillée	Oui	☐	Non	☐	De temps à temps	☐
L'eau de notre puits privé	Oui	☐	Non	☐	De temps à temps	☐
L'eau du puits de notre village	Oui	☐	Non	☐	De temps à temps	☐
L'eau de la rivière	Oui	☐	Non	☐	De temps à temps	☐
L'eau du lac	Oui	☐	Non	☐	De temps à temps	☐
Autre (à préciser)	_____					
Facteurs de risque : Pratiques (2) – Hygiène du corps						
Vous baignez-vous le corps?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, où vous baignez-vous ? (cochez une ou plusieurs cases)						
A la maison avec l'eau du robinet	Oui	☐	Non	☐	De temps à temps	☐
A la maison avec l'eau de notre puits	Oui	☐	Non	☐	De temps à temps	☐
Avec l'eau du puits du village	Oui	☐	Non	☐	De temps à temps	☐
A la rivière	Oui	☐	Non	☐	De temps à temps	☐
Au lac	Oui	☐	Non	☐	De temps à temps	☐
A la source aménagée du village	Oui	☐	Non	☐	De temps à temps	☐
Autre place (préciser)	_____					

QUESTIONNAIRE INDIVIDUEL (suite)						
Hygiène des vêtements: Pratiques (2) – Eau de lessive, pêche, travaux de champs						
Lavez-vous vos vêtements ? Oui ☐ Non ☐ De temps à temps ☐ (cochez une ou plusieurs cases)						
Si oui, où les lavez-vous ? (cochez une ou plusieurs cases)						
Avec l'eau du robinet de la maison	Oui	☐	Non	☐	De temps à temps	☐
A la source aménagée du village	Oui	☐	Non	☐	De temps à temps	☐
Avec l'eau de notre puits privé	Oui	☐	Non	☐	De temps à temps	☐
Avec l'eau du puits de notre village	Oui	☐	Non	☐	De temps à temps	☐
A la rivière	Oui	☐	Non	☐	De temps à temps	☐
Au lac	Oui	☐	Non	☐	De temps à temps	☐
Autre (à préciser)	_____					
Facteurs de risque : Pratiques (3) – Autres contacts à risques						
A part le bain et la lessive, quelle autre activité faites-vous dans l'eau de la rivière ou du lac ?						
Faites-vous de la pêche ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, à quelle fréquence ? Quotidienne ☐ Combien de fois par semaine ?_____						
Autre fréquence (préciser)						
Faites-vous les travaux de champ ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, avez-vous des jambières ?	Oui	☐	Non	☐		
Portez-vous ces jambières au champ ?	Oui	☐	Non	☐	De temps à temps	☐

QUESTIONNAIRE INDIVIDUEL (suite)						
Facteurs de risque : Pratiques (4) – Hygiène des mains						
Lavage des mains (cochez une ou plusieurs cases)						
Lavez-vous vos mains ?	Oui	☐	Non	☐	De temps à temps	☐
Au savon ?	Oui	☐	Non	☐	De temps à temps	☐
A la cendre ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, quand vous lavez-vous les mains ?						
Avant de manger	Oui	☐	Non	☐	De temps à temps	☐
Après avoir été en toilette	Oui	☐	Non	☐	De temps à temps	☐
Après avoir changé les couches de bébé	Oui	☐	Non	☐	De temps à temps	☐
Autre (préciser) _____	Oui	☐	Non	☐	De temps à temps	☐
Autre (à préciser)	_____					
Facteurs de risque : Pratiques (5) – Port de chaussures						
A -vous de chassures (fermées) ?	Oui	☐	Non	☐	Sandales	☐
Si oui, les portez-vous ? (cochez une ou plusieurs cases)						
Chaque fois que vous sortez ?	Oui	☐	Non	☐	De temps à temps	☐
Si quelques fois, à quelles occasions les portez-vous ? (cochez une ou plusieurs cases)						
Aller à l'église (mosquée)	Oui	☐	Non	☐	De temps à temps	☐
Aller au marché	Oui	☐	Non	☐	De temps à temps	☐
Aller à l'école	Oui	☐	Non	☐	De temps à temps	☐
Autre occasion (préciser)	_____					

QUESTIONNAIRE INDIVIDUEL (suite)						
Facteurs de risque : Pratiques (6) – Hygiène alimentaire						
Comment avez-vous l'habitude de manger vos aliments ? (cochez une ou plusieurs cases)						
Crus ?	Oui	☐	Non	☐	De temps à temps	☐
Cuits ?	Oui	☐	Non	☐	De temps à temps	☐
Autre (préciser) _____						
Si crus, quels sont les aliments que vous mangez crus ? Citez-les _____						
Les lavez-vous avant de manger ?	Oui	☐	Non	☐	De temps à temps	☐
Après avoir été en toilette	Oui	☐	Non	☐	De temps à temps	☐
Après avoir changé les couches de bébé	Oui	☐	Non	☐	De temps à temps	☐
Autre (préciser) _____	Oui	☐	Non	☐	De temps à temps	☐
Autre (à préciser)	_____					
Facteurs de risque : Pratiques (7) – Autres habitudes dommageables pour la santé						
Consommez-vous de l'alcool ou autres drogues ? (cochez une ou plusieurs cases)						
Chaque fois que vous sortez ?	Oui	☐	Non	☐	De temps à temps	☐
Si quelques fois, à quelles occasions les portez-vous ? (cochez une ou plusieurs cases)						
Alcool ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, depuis combien de temps ? _____						
Tabac ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, depuis combien de temps ? _____						
Chanvre ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, depuis combien de temps ? _____						
Essence ?	Oui	☐	Non	☐	De temps à temps	☐
Si oui, depuis combien de temps ? _____						
Autre drogue ? (préciser)	Oui	☐	Non	☐	De temps à temps	☐
Si oui, depuis combien de temps ? _____						
Facteurs de risque : Pratiques (8) – Durée de séjour au village						
Depuis combien de temps résidez-vous ici ? _____ (années)						

QUESTIONNAIRE INDIVIDUEL (suite)						
Connaissances (1) Laisser la personne répondre librement et cocher ses réponses :						
Connaissance sur la bilharziose comme maladie (cochez une ou plusieurs cases)						
Avez-vous entendu parler de la bilharziose ?	Oui	☐	Non	☐		
Si oui, par quel canal de communication ?						
Par la radio ?	Oui	☐	Non	☐		
En lisant un journal (livre)	Oui	☐	Non	☐		
Par les parents	Oui	☐	Non	☐		
A l'école	Oui	☐	Non	☐		
A l'église	Oui	☐	Non	☐		
A la mosquée	Oui	☐	Non	☐		
Par un ami _____	Oui	☐	Non	☐		
Par un autre moyen (à préciser)	_____					
Si oui, comment ses manifeste-t-elle ? (signes et symptômes)						
Par la diarrhée	Oui	☐	Non	☐	Je ne sais pas	☐
Selles avec du sang	Oui	☐	Non	☐	Je ne sais pas	☐
Mucus dans les selles	Oui	☐	Non	☐	Je ne sais pas	☐
Gros foie	Oui	☐	Non	☐	Je ne sais pas	☐
Grosse rate	Oui	☐	Non	☐	Je ne sais pas	☐
Ventre rempli de liquide	Oui	☐	Non	☐	Je ne sais pas	☐
Vomissement de sang	Oui	☐	Non	☐	Je ne sais pas	☐
Autre signe ou symptôme (préciser)	_____					
Quelle en est la cause ? (Cause)						
Je ne sais pas ☐						
Sorcellerie	Oui	☐	Non	☐	Je ne sais pas	☐
Microbes	Oui	☐	Non	☐	Je ne sais pas	☐
Vers intestinaux	Oui	☐	Non	☐	Je ne sais pas	☐
Autre vers (préciser)	_____					
Autre cause (préciser)	_____					

Comment contracte-t-on la bilharziose ? (Transmission)						
Je ne sais pas ☐						
En buvant de l'eau contaminée	Oui	☐	Non	☐	Je ne sais pas	☐
En jouant ou en travaillant dans l'eau	Oui	☐	Non	☐	Je ne sais pas	☐
En marchant pieds nus	Oui	☐	Non	☐	Je ne sais pas	☐
Par le tatouage	Oui	☐	Non	☐	Je ne sais pas	☐
En saluant les personnes atteintes	Oui	☐	Non	☐	Je ne sais pas	☐
Par les relations sexuelles	Oui	☐	Non	☐	Je ne sais pas	☐
Autre mode de transmission (préciser)	_____					
Comment peut-on éviter la bilharziose ? (Prévention)						
Je ne sais pas ☐						
En buvant de l'eau potable	Oui	☐	Non	☐	Je ne sais pas	☐
En ne jouant ni travaillant dans l'eau	Oui	☐	Non	☐	Je ne sais pas	☐
En portant des jambières en cultivant	Oui	☐	Non	☐	Je ne sais pas	☐
En portant des chaussures fermées	Oui	☐	Non	☐	Je ne sais pas	☐
En lavant les vêtements dans une eau saine	Oui	☐	Non	☐	Je ne sais pas	☐
En utilisant les préservatifs	Oui	☐	Non	☐	Je ne sais pas	☐
Autre mode de prévention (préciser)	_____					
Comment traite-t-on la bilharziose ? (Traitement)						
Je ne sais pas ☐						
En prenant le Praziquantel (Biltricide)	Oui	☐	Non	☐	Je ne sais pas	☐
En prenant le mebendazole ou l'albendazole	Oui	☐	Non	☐	Je ne sais pas	☐
Autre mode de médicament (préciser)						
Attitudes et histoire familiale – 1 (laisser la personne répondre librement et cocher ses réponses)						
Y a-t-il quelqu'un de votre famille qui a été diagnostiqué de bilharziose	Oui	☐	Non	☐	Je ne sais pas	☐
Si oui, qu'avez-vous fait pour lui ? (Prise en charge)						
Rien ☐						
Médicaments traditionnels	Oui	☐	Non	☐	Je ne sais pas	☐
Aller consulter les tradi-praticiens	Oui	☐	Non	☐	Je ne sais pas	☐
Acheter les médicaments à la pharmacie	Oui	☐	Non	☐	Je ne sais pas	☐

Aller au Centre de santé	Oui	☐	Non	☐	Je ne sais pas	☐

Cas de décès dû à la bilharziose ? (Histoire familiale – 2)						
Y a-t-il eu quelqu'un de votre famille mort de bilharziose ?	Oui	☐	Non	☐	Je ne sais pas	☐
Je ne sais pas ☐						
Pensez-vous que la bilharziose est un grand problème dans votre village ?	Oui	☐	Non	☐	Je ne sais pas	☐
Si oui, que devriez-vous faire pour lutter contre la bilharziose ? (Réponse libre)						
_____						
MERCI D'AVOIR REPONDU A NOS QUESTIONS						

Household Questionnaire

ENQUÊTE SCHISTOSOMIASE EN ITURI
QUESTIONNAIRE MENAGE

Date_____/_____/_____

Numéro d'identification (ID) (ménage) |____| |____| |____|

Village (nom)_____

Chef du ménage

Nom et postnom _____

Homme □ Femme □

Habitation :

- Mur – :

En paille ou en feuilles □
En terre battue □ Crépi □ Non crépi □ Avec peinture □
En pierre □ Crépi □ Non crépi □ Avec peinture □
En briques adobe non-cuites □ Crépi □ Non crépi □ Avec peinture □
En briques adobe cuites □ Crépi □ Non crépi □ Avec peinture □
En briques pressées cuites □ Crépi □ Non crépi □ Avec peinture □
En monobloc en ciment □ Crépi □ Non crépi □ Avec peinture □

- Plancher – :

En terre battue □ Pavé en argile □ Non pavé □
En briques adobe non-cuites □
En briques adobe cuites □
En briques pressées cuites □
Pavé en ciment □ Avec peinture □ Sans peinture □
En carreaux □
En granitos coulés □

- Toit – :

En chaume ou en feuilles □
En bâche □
En tôles ondulées BWG 30 – 36 □
En tuiles
En ardoise
En tôles ondulées BWG 20 – 28 □

Distance par rapport au plan d'eau le plus proche _____

Revenu annuel moyen du ménage (estimation) : _____ USD

Elevage au ménage :
Aucun □
Poules ? Oui □ Non □ Combien ? _____
Canard ? Oui □ Non □ Combien ? _____
Chèvres ? Oui □ Non □ Combien ? _____

Brebis ? Oui □ Non □ Combien ? _____

Porcs ? Oui □ Non □ Combien ? _____

Chiens ? Oui □ Non □ Combien ? _____

Chats ? Oui □ Non □ Combien ? _____

Vaches ? Oui □ Non □ Combien ? _____

Autre animal (préciser) Oui □ Non □ Combien ? _____

Objet possédé :

Aucun

Vélo Oui □ Non □ Combien ? _____

Machine à coudre Oui □ Non □ Combien ? _____

Frigo Oui □ Non □ Combien ? _____

Congélateur Oui □ Non □ Combien ? _____

Moto Oui □ Non □ Combien ? _____

Motopompe Oui □ Non □ Combien ? _____

Voiture Oui □ Non □ Combien ? _____

Camion Oui □ Non □ Combien ? _____

Autre (à préciser) _____ Oui □ Non □ Combien ? _____

Facteurs de risque :

- Accès à l'eau potable –

Où vous approvisionnez-vous en eau potable ?

L'eau du robinet de la maison □

L'eau embouteillée □

L'eau de notre puits privé □

L'eau de la source aménagée du village □ Distance _____ km

L'eau du puits du village □ Distance _____ km

L'eau de la rivière □ Distance _____ km

L'eau du lac □ Distance _____ km

Autre eau (préciser) _____ Distance _____ km

- Assainissement –

Latrine dans votre parcelle ? Présence (vérifier) Oui □ Non □

Si latrine présente, vérifier qualité de la latrine :

Plancher Mauvaise □ Bonne □

Porte Mauvaise □ Bonne □

Profondeur Mauvaise □ Bonne □

Propreté orifice Mauvaise □ Bonne □

Mouches Mauvaise □ Bonne □

Distance < 10 m = Mauvaise □ > 10 m = Bonne □

Utilisation – L'utilisez-vous ? Oui □ Non □ Quelques fois □

Quand la dernière fois avez-vous utilisé la latrine ? _____

Si vous n'utilisez pas de latrine, Où allez-vous faire vos besoins ?

Derrière la maison □ Oui □ Non □ Quelques fois □

Dans la brousse proche □ Oui □ Non □ Quelques fois □

Dans la rivière □ Oui □ Non □ Quelques fois □

Dans le lac □ Oui □ Non □ Quelques fois □

Dans le seau □ Oui □ Non □ Quelques fois □

Dans les sachets Oui □ Non □ Quelques fois □

Autre place (préciser) _____

Trou à ordures :

Présence : Absent □ Présent □

Qualité : Mauvaise □ Bonne □

Quel est, selon vous, le plus grand problème de santé de votre village ?

_____

Selon vous, qu'est-ce qui doit être fait pour résoudre ce problème dans votre village ?

_____

Par qui ? _____

Comment ? _____

A quel moment (Quand) ? _____

Par quel moyen ? _____

Individual form

ENQUÊTE SCHISTOSOMIASE EN ITURI

Formulaire individuel

Date_____/_____/_____

Numéro d'identification _____

Village _____

Données démographiques

Nom et postnom _____

Date de naissance : le _____/ _____/ _____

Age _____ Sexe _____ N

Statu matrimonial : Célibataire □ Marié □ Veuf (ve) □ Divorcé □

Nationalité _____ Tribu _____

Données anthropométriques

Poids _____ kg Taille _____ cm BMI _____

Données parasitologiques

a) Examen de selles	Œufs par lame	Œufs par gramme (œufs/g)=epg	Seuil de sévérité de linfection (modérée/massive)	Infection modérée/massive	
Schistosoma mansoni			≥ 100 epg		
Ascaris lumbricoides			≥ 5000 epg		
Necator americanus (ankylostome)			≥ 2000 epg		
Trichuris trichiura			≥ 1000 epg		
Strongyloides stercoralis			NA		
Enterobius vermicularis			NA		

Autres parasites identifiés (préciser)

b) Urine : examen visuel	Présence	
	Oui	Non
Hématurie visible		
Microhématurie (au moyen d'une bandelette réactive)		

c) Urine : examen microscopique	Œufs par 10 ml d'urine	Seuil d'infection massive	Infection massive (oui / non)	
Schistosoma haematobium		≥ 350 œufs/10 ml		
d) Circulating Cathodic Antigen (CCA) (Positif/Négatif)				

School form

ENQUÊTE SCHISTOSOMIASE ET CO-INFECTIONS EN MILIEU SCOLAIRE
FORMULAIRE D'ÉCOLE

Ecole_____
Date_____/_____/_____

Territoire_____
Zone de santé_____

Effectif
Nombre total d'élèves _____
Nombre de filles _____

Nombre de classes _____
Nombre d'enseignants _____

Eau
L'école est-elle approvisionnée en eau ? Oui □ Non □
Nature de l'approvisionnement en eau
_____
Existe-t-il des sources d'eau à proximité de l'école ? Oui □ Non □
Nature de ces sources

Assainissement
L'école possède-t-elle des latrines ? Oui □ Non □
Etat des latrines : Bon □ Mauvais □ ou

Services sanitaires
Etablissement de soins le plus proche (nom) :
_____
Type _____
Distance _____ km

Traitement
Nombre d'enfants scolarisés traités depuis les trois derniers mois pour une géo-helminthiase :
_____
Nombre d'enfants scolarisés traités depuis les six derniers mois pour une schistosomiase :
_____
Nombre d'enfants scolarisés traités au courant du mois pour un paludisme :
_____
Nombre d'enfants scolarisés traités au courant de la période pour une infection (à préciser) :
_____

Village form

ENQUÊTE SCHISTOSOMIASE ET CO-INFECTIONS EN MILIEU RURAL
FORMULAIRE DU VILLAGE

Village _____
Date_____/_____/_____

Territoire_____
Zone de santé_____

Effectif
Nombre total d'habitants _____
Nombre de femmes _____

Nombre d'enfants d'âge scolaire _____
Nombre d'enfants d'âge préscolaire _____

Eau
Le village est-il approvisionné en eau ? Oui □ Non □
Nature de l'approvisionnement en eau
_____
Existe-t-il des sources d'eau à proximité du village ? Oui □ Non □
Nature de ces sources _____

Assainissement
Le village possède-t-il des latrines (village assaini) ? Oui □ Non □
Etat des latrines : Bon □ Mauvais □ ou _____

Services sanitaires
Etablissement de soins le plus proche (nom) :
_____
Type _____
Distance _____ km

Traitement
Nombre de personnes traitées depuis les trois derniers mois pour une géo-helminthiase :
_____
Nombre de personnes traitées depuis les six derniers mois pour une schistosomiase :
_____
Nombre de personnes traitées au courant du mois pour un paludisme :
_____
Nombre de personnes traitées au courant de la période pour une infection (à préciser) :
_____