Aus dem Institut für Tropenmedizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

# Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy

Zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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von

Olga Lilith Staudacher

aus Ravensburg

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## Inhaltsverzeichnis

## Abstract in English

Objectives. Preventive chemotherapy of schoolchildren against soil-transmitted helminths (STHs) is widely implemented in Rwanda. However, data on its actual efficacy are lacking. We assessed prevalence, associated factors, and manifestation of STH infection among schoolchildren in southern highland Rwanda as well as cure and reinfection rates.

Methods. 622 children (rural, 301; urban, 321) were included preceding the administration of a single dose of 500 mg mebendazole. Before treatment, and after two and fifteen weeks, STH infection was determined by Kato-Katz smears and by PCR assays for *Ascaris lumbricoides*. Clinical and anthropometric data, socioeconomic status and factors potentially associated with STH infection were assessed.

Results. STH infection was present in 38% and 13% of rural and urban schoolchildren, respectively. *A. lumbricoides* accounted for 96% of infections. Of these, one third was detected by PCR exclusively. Factors associated with STH infection differed greatly between rural and urban children. Likewise, STH infection was associated with stunting and anaemia only among urban children. The cure rate after two weeks was 92%. Among eight non-cleared *A. lumbricoides* infections, seven were submicroscopic. Reinfection within three months occurred in 7%, but the rate was increased among rural children, and with initially present infection, particularly at comparatively high intensity.

Conclusions. The rural-urban difference in factors associated with STH infection and in reinfection rates highlight the need for targeted interventions to reduce transmission. PCR assays may help in detecting low level infections persisting after treatment. In southern Rwanda, mebendazole is highly effective against the STH infectionspredominated by *A. lumbricoides.*

# Abstrakt auf Deutsch


Ziele. Eine präventive medikamentöse Therapie gegen Geohelminthen, so genannten Soil Transmitted Helminths (STHs), ist bereits seit einiger Zeit flächendeckend in Ruanda implementiert. Dennoch fehlen Daten über deren Effektivität. Unsere Studie untersuchte Prävalenz, assoziierte Faktoren und die Verbreitung von STH-Infektionen bei Schulkindern in Südruanda. Heilungs- und Reinfektionsrate wurden evaluiert.

Methoden. 622 Kinder wurden in die Studie eingeschlossen, davon 301 in ländlicher und 321 in urbaner Umgebung. Ihnen wurde einmalig unter Aufsicht 500mg Mebendazol verabreicht. Vor der Behandlung wurde von jeder Probandin und jedem Probanden je eine Stuhlprobe untersucht. Dies geschah durch Kato-Katz-Technik auf STHs und zusätzlich durch eine PCR auf Infektion mit *Ascaris lumbricoides.* Die Untersuchung wurden zwei und fünfzehn Wochen nach der Mebendazoleinnahme wiederholt. Anthropometrische Daten, der familiäre sozio-ökonomische Status sowie Risikofaktoren mit möglicher Assoziation zu STHs wurden parallel evaluiert.

Ergebnisse. Die initiale Prävalenz von STH-Infektionen lag auf dem Land bei 38%, in der Stadt bei 13%. In 96% der Fälle handelte es sich um Infektionen mit *A. lumbricoides*. Ein Drittel dieser konnte ausschließlich durch eine PCR entdeckt werden. Die mit einer Infektion assoziiert Faktoren variierten zwischen ruralem und urbanem Milieu deutlich. Nur in der städtischen Schule bestand eine Assoziation von Wurmeiausscheidung und Minderwuchs bzw. Anämie. Die Heilungsrate betrug 92%. Von acht persistierenden *A. lumbricoides*-Infektionen waren sieben submikroskopisch.

Eine Reinfektion nach drei Monaten trat bei 7% der Kinder auf. Der Anteil war unter SchülerInnen der ländlichen Schule und solchen, die schon initial unter einer Infektion gelitten hatten, höher. Besonders häufig kam es zur Reinfektion, wenn bei der initialer Untersuchung die Anzahl von im Stuhl ausgeschiedenen Eiern vergleichsweise hoch gewesen war.

Schlussfolgerung. Die mit STH-Infektion assoziierten Faktoren variieren zwischen den verschiedenen Umgebungen. Auch die Reinfektionsraten unterscheiden sich. Zur Minimierung der Übertragungshäufigkeit ist also eine gezielte Intervention notwendig. PCR-Assays können eingesetzt werden, um Infektionen zu erkennen, die in geringer Intensität auch nach einer Behandlung persistieren. Im südlichen Ruanda ist die Therapie mit Mebendazol gegen STH-Infektionen, besonders gegen die vorherrschende Spezies *A. lumbricoides,* überaus effizient.

## Eidesstattliche Versicherung

„Ich, Olga Lilith Staudacher, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: "Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
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## Ausführliche Anteilserklärung an der erfolgten Publikation

Publikation: Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy.
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Beitrag im Einzelnen:
Akquirieren des Studienpersonals vor Ort, Mitkonzeption der Abläufe in den bereitgestellten Räumlichkeiten, Einschluss der ProbandInnen, Materialbeschaffung, Probensammlung, Kommunikation mit örtlichen Behörden und zwischen Studienbeteiligten, Buchhaltung, Probenuntersuchung und -konservierung, Konzeption des Fragebogens und dessen Verteilung, Dateneingabe und -auswertung, Journalartikel

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| Mark | Rank | Abbreviated Journal Title *(linked to journal information)* | ISSN | JCR Data | | | | | | Eigenfactor® Metrics | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total Cites | Impact Factor | 5-Year Impact Factor | Immediacy Index | Articles | Cited Half-life | *Eigenfactor® Score* | *Article Influence® Score* |
| ☐ | 1 | PLOS NEGLECT TROP D | 1935-2735 | 8527 | 4.489 | 4.976 | 0.608 | 605 | 2.9 | 0.04015 | 1.498 |
| ☐ | 2 | MALARIA J | 1475-2875 | 8286 | 3.489 | 3.628 | 0.569 | 457 | 3.7 | 0.02954 | 0.975 |
| ☐ | 3 | AM J TROP MED HYG | 0002-9637 | 19670 | 2.736 | 2.947 | 0.699 | 382 | 9.5 | 0.02875 | 0.917 |
| ☐ | 4 | ACTA TROP | 0001-706X | 5653 | 2.519 | 2.710 | 0.776 | 223 | 7.6 | 0.01067 | 0.768 |
| ☐ | 5 | TROP MED INT HEALTH | 1360-2276 | 6600 | 2.302 | 2.953 | 0.410 | 166 | 6.8 | 0.01644 | 1.031 |
| ☐ | 6 | T ROY SOC TROP MED H | 0035-9203 | 8411 | 1.931 | 2.453 | 0.294 | 126 | >10.0 | 0.01065 | 0.769 |
| ☐ | 7 | MEM I OSWALDO CRUZ | 0074-0276 | 5757 | 1.566 | 1.961 | 0.168 | 185 | 9.0 | 0.00861 | 0.539 |
| ☐ | 8 | ANN TROP MED PARASIT | 0003-4983 | 2765 | 1.203 | 1.453 | | 0 | >10.0 | 0.00229 | 0.414 |
| ☐ | 9 | ANN TROP PAEDIATR | 0272-4936 | 729 | 1.173 | 1.117 | | 0 | >10.0 | 0.00137 | 0.434 |
| ☐ | 10 | REV SOC BRAS MED TRO | 0037-8682 | 2027 | 0.942 | 0.947 | 0.105 | 152 | 7.2 | 0.00325 | 0.224 |
| ☐ | 11 | ASIAN PAC J TROP MED | 1995-7645 | 560 | 0.926 | 0.665 | 0.172 | 192 | 2.4 | 0.00179 | 0.135 |
| ☐ | 12 | REV INST MED TROP SP | 0036-4665 | 1643 | 0.907 | 1.213 | 0.120 | 75 | >10.0 | 0.00170 | 0.309 |
| ☐ | 13 | J TROP PEDIATRICS | 0142-6338 | 1474 | 0.857 | 1.196 | 0.172 | 87 | 8.5 | 0.00262 | 0.382 |
| ☐ | 14 | PATHOG GLOB HEALTH | 2047-7724 | 70 | 0.841 | 0.841 | 0.194 | 62 | | 0.00036 | 0.309 |
| ☐ | 15 | TROP BIOMED | 0127-5720 | 591 | 0.816 | 1.045 | 0.059 | 85 | 5.0 | 0.00148 | 0.267 |
| ☐ | 16 | BIOMEDICA | 0120-4157 | 526 | 0.617 | 0.760 | 0.106 | 85 | 6.2 | 0.00108 | 0.202 |
| ☐ | 17 | LEPROSY REV | 0305-7518 | 550 | 0.587 | 0.781 | 0.000 | 38 | 9.5 | 0.00056 | 0.182 |
| ☐ | 18 | SE ASIAN J TROP MED | 0125-1562 | 2715 | 0.546 | | 0.045 | 111 | >10.0 | 0.00310 | |
| ☐ | 19 | TROP DOCT | 0049-4755 | 697 | 0.528 | 0.576 | 0.100 | 50 | 7.5 | 0.00148 | 0.190 |
| ☐ | 20 | J VENOM ANIM TOXINS | 1678-9199 | 287 | 0.426 | 0.435 | 0.065 | 31 | 6.1 | 0.00056 | 0.103 |

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# Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy

**Olga Staudacher[1], Jakob Heimer[1], Florian Steiner[1], Yvette Kayonga[2], Jean M. Havugimana[3], Ralf Ignatius[1], Andre Musemakweri[3], Fidele Ngabo[4], Gundel Harms[1], Jean-Bosco Gahutu[3] and Frank P. Mockenhaupt[1]**

1 *Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Berlin, Germany*
2 *Faculty of Social Work, Catholic University of Rwanda, Huye, Rwanda*
3 *University Teaching Hospital of Butare, University of Rwanda, Butare, Rwanda*
4 *Maternal and Child Health Unit, Ministry of Health, Kigali, Rwanda*

**Abstract**

OBJECTIVES Preventive chemotherapy of schoolchildren against soil-transmitted helminths (STHs) is widely implemented in Rwanda. However, data on its actual efficacy are lacking. We assessed prevalence, associated factors and manifestation of STH infection among schoolchildren in southern highland Rwanda as well as cure and reinfection rates.

METHODS Six hundred and twenty-two children (rural, 301; urban, 321) were included preceding the administration of a single dose of 500 mg mebendazole. Before treatment, and after 2 and 15 weeks, STH infection was determined by Kato-Katz smears and by PCR assays for *Ascaris lumbricoides*. Clinical and anthropometric data, socio-economic status and factors potentially associated with STH infection were assessed.

RESULTS Soil-transmitted helminth (STH) infection was present in 38% of rural and in 13% of urban schoolchildren. *Ascaris lumbricoides* accounted for 96% of infections. Of these, one-third was detected by PCR exclusively. Factors associated with STH infection differed greatly between rural and urban children. Likewise, STH infection was associated with stunting and anaemia only among urban children. The cure rate after 2 weeks was 92%. Among eight non-cleared *A. lumbricoides* infections, seven were submicroscopic. Reinfection within 3 months occurred in 7%, but the rate was higher among rural children, and with initially present infection, particularly at comparatively high intensity.

CONCLUSIONS The rural–urban difference in factors associated with STH infection and in reinfection rates highlights the need for targeted interventions to reduce transmission. PCR assays may help in detecting low-level infections persisting after treatment. In southern Rwanda, mebendazole is highly effective against the STH infections predominated by *A. lumbricoides*.

**keywords** Soil-transmitted helminths, *Ascaris*, mebendazole, cure rate, reinfection, Rwanda

## Introduction

The soil-transmitted helminths (STHs) *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (*Ancylostoma duodenale*, *Necator americanus*) infect more than 1 billion individuals worldwide, and are regarded as one of the world's most important causes of reduced physical and cognitive development. In that process, malabsorption and malnutrition associated with chronic infection are central mechanisms, especially in children (Bethony *et al.* 2006; World Health Organisation 2006; Hotez *et al.* 2007). While direct mortality is comparatively low, the loss in disability-adjusted life years is enormous (World Health Organisation 2008).

The central element in the global strategy of STH control is preventive chemotherapy administering single doses of mebendazole or albendazole once or twice yearly to risk groups, for example schoolchildren (World Health Organisation 2006). Such regular treatment of schoolchildren aims at reducing the worm burden below the threshold associated with morbidity. In fact, regular deworming has been shown to improve growth and physical fitness, cognitive performance, and school attendance (Bethony *et al.* 2006). However, there is concern that the roll-out of preventive chemotherapy promotes the emergence of drug resistance as observed in veterinary medicine (Wolstenholme *et al.* 2004). Yet, while a meta-analysis reported high cure rates of single dose mebendazole and albendazole for *A. lumbricoides* (close to or above 90%), mebendazole cure rates were only 36% for *T. trichiura* and 15% for hookworm (Keiser & Utzinger 2008). Reinfection within 3 months of deworming occurs on average in one of three treated children (Jia *et al.* 2012). Therefore, additional preventive measures need to tackle the determinants of STH transmission, which include poverty, poor sanitation and water supply, overcrowding, lack of education and limited access to health care (Bethony *et al.* 2006; Brooker *et al.* 2006; Ziegelbauer *et al.* 2012). Transmission frequently differs between rural and urban settings, which may stem from disparities in population density, sanitation, behaviour, etc. However, the actual factors are insufficiently established so far (Brooker *et al.* 2006; Amoah *et al.* 2007). Also, submicroscopic STH infections, detectable by PCR only, are frequent in individuals deemed non-infected by standard microscopy (Basuni *et al.* 2011; Jonker *et al.* 2012), but their actual role in STH epidemiology and control is unclear.

In Rwanda, a national survey in 2008 among more than 8000 schoolchildren in 30 districts found an overall STH prevalence of 66%. In the southern district of Huye, this figure was 72%, with *A. lumbricoides* (prevalence, 61%) dominating over hookworm (31%) and *T. trichiura* (13%) (Center for Infectious Disease Control Rwanda 2008). In response to that situation, Rwanda during 3 years administered some 18 million STH treatments corresponding to at least 4.2 million individuals, that is nearly half of the country's population. A repeat survey after 1 year found a reduction in STH prevalence by 14% (hookworm, −72%) (Ruxin & Negin 2012), but actual efficacy data are lacking. In 2010, one-third of pre-school children in Huye district villages were STH infected (Ignatius *et al.* 2012), possibly indicating a further reduction since the implementation of the nationwide deworming campaign.

Here we assessed the prevalence of STH infection in schoolchildren from a rural and an urban setting in Huye district, southern Rwanda, including sensitive PCR assays for *A. lumbricoides* and aiming at estimating associated factors, clinical characteristics, cure rates of regular school-based deworming using mebendazole, and reinfection rates during 3 months of follow-up.

## Materials and methods

### Study population

This observational, repeated cross-sectional study was conducted from May to September 2012 in the area of Butare, Huye district, southern province of Rwanda, alongside the national deworming campaign. Butare (population approximately 100 000; altitude 1768 m) is located on the country's central plateau (average altitude, 1800 m; yearly rainfall, 1200 mm; mean temperature, 19 °C) and is surrounded by densely populated farmland hills. Two primary schools in the district were purposely selected as study sites, namely in a rural (Gatovu; 18 km out of town; 708 schoolchildren) and an urban (Ngoma, 547 schoolchildren) setting. Information on the study purpose and procedures was provided during meetings at both schools and questions answered to parents and teachers. A pragmatic number of 400 children each were invited to participate. This number was determined by the laboratory capacity of stool microscopy. Informed written consent was obtained from parents or guardians, and additional assent of students of 8 years or older. The study was approved by the Rwanda National Ethics Committee (RNEC 105/RNEC/2012) and by the Rwandan Ministry of Education (MINEDUC/S&T/0079/2012).

### Sample collection and examinations

The study schedule comprised an examination on the day of routine bi-annual school-based preventive chemotherapy and preceding the administration of a single dose of 500 mg mebendazole (Micro Labs Ltd., Hosur, India) as well as identical examinations 2 and 15 weeks after deworming. Children were provided with pre-labelled containers and asked to bring a fresh stool sample at the scheduled dates. At study initiation, a venous blood sample was collected.

Age, sex, weight, height (statometer Seca 213, Seca, Germany), and axillary temperature were documented. Age was verified with birth certificates and school documents. Underweight and stunting were defined as a weight-for-age z (WAZ) score and a height-for-age z (HAZ) score of <–2 standard deviations (SD) following calculation by WHO Anthro Plus (http://www.who.int/growthref/tools/en/). Children were clinically examined by the study physician (JMH), and treatment was provided according to Rwandan treatment guidelines if needed.

### Socio-economic status

Trained health workers performed interviews with household heads at their homes or at school using pre-tested questionnaires in Kinyarwanda to assess the socio-economic status (SES) of the children's families as well as characteristics and behaviour proximately or possibly related to STH infection (the English version of the questionnaire is available from the authors on request). Parameters assessed included information on maternal and paternal education and occupation, household assets, ability to pay school fees and health insurance, ownership, type, and number of livestock, waste disposal, toilet facilities and hygiene measures, water use, eating habits as well as knowledge, attitudes, and practice with regard to helminth infection, treatment, and prevention.

### Laboratory examinations

The stool samples were processed and analysed on the day of collection. Presence and intensity of STH infection were estimated using the Kato-Katz technique (Word Health Organisation 1994). Single Kato-Katz thick smears were prepared using standard 41.7 mg stool templates (Vestergaard Frandsen, Lausanne, Switzerland); eggs were microscopically counted and recorded for each species separately. Faecal egg counts (FEC) were estimated and transformed into eggs per gram (epg) (Word Health Organisation 1994). Ten per cent of samples were cross-checked by microscopy following formalin-ethyl acetate centrifugation. In addition, infections with *A. lumbricoides* were detected by a real-time PCR assay using published primers (Basuni *et al.* 2011). For that, aliquots of stool were stored at −70 °C and transported on dry ice to Berlin. DNA was extracted by commercial kits (Qiamp DNA Stool Mini Kit, Qiagen, Hilden, Germany). For each sample, phocine herpesvirus 1 (PhHV-1, kindly provided by Dr. Martin Schutten, Department of Virology, Erasmus Medical Centre, Rotterdam, The Netherlands) was added to the isolation lysis buffer to serve as an internal control for the extraction process (Niesters 2002). Real-time PCR assays were run on a Lightcycler 480 (Roche Diagnostics) including positive and negative controls. Cycle threshold (Ct) values of >36 were considered to reflect limited reproducibility due to low copy numbers, and for all samples yielding a Ct-value of >36, assays were repeated. No sample had to be excluded because of evidence of faecal inhibitory factors (Ct-value for the internal control PhHV-1 >36). A stool sample was considered positive for STHs when either the Kato-Katz smear or the *Ascaris*-specific PCR assay yielded a positive result; a positive PCR result for *Ascaris* but a negative one by microscopy was considered to reflect a submicroscopic infection. For all helminths species, infection intensity was graded as low, moderate and high corresponding to FECs of 1 <5000, 5000 <50000 and ≥50000 epg for *A. lumbricoides*; 1 <1000, 1000 <10000 and ≥10000 epg for *T. trichiura*; and 1 <2000, 2000 <4000 and ≥4000 epg for hookworms, respectively (Montresor *et al.* 1998).

Haemoglobin (Hb) concentrations were measured by a HemoCue photometer (Angelholm, Sweden) and anaemia defined according to age-related and altitude-adjusted cut-off values (Sullivan *et al.* 2008). *Plasmodium falciparum* and non-falciparum malaria parasites were detected by immunochromatographic dipstick tests (Malaria Total Qick Test, Cypress Diagnostics, Langdorp, Belgium).

### Statistical analysis

Of 712 participating children, 662 (93%) provided a pretreatment stool sample, and 622 (87%) samples were available for analysis by both microscopy and *A. lumbricoides* PCR (40 samples were lost during processing or transport, or the sample volume was too low for subsequent PCR). The data of these 622 children form the basis for the present analysis. Data were double-entered and cross-checked. Data analysis was performed using Statview 5.0 (SAS Institute Inc.). Continuous variables were compared between groups by the non-parametric Mann–Whitney or Kruskal–Wallis tests, and proportions by $\chi^2$ test or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed. Evaluation of independent predictors of, for example STH infection, was performed by multivariate logistic regression analysis including factors found to be univariately associated at a level of $P < 0.10$. Stepwise backward selection was performed, and final models included those factors that retained statistical significance ($P < 0.05$). Cure rate (CR) was calculated as the proportion of children positive for STHs at the baseline survey who became negative 14 days post-treatment.

## Results

The baseline characteristics of the 622 children (301 rural, 321 urban) are shown in Table 1. As compared to their urban counterparts, children attending the rural primary school were slightly older. More common observations among rural children included poor nutritional status, reduced appetite, any clinical finding, skin infections, clinically assessed malnutrition, dental caries and a positive malaria test although Hb levels were slightly higher. Results of pulmonary and cardiac auscultation and of abdominal and neurological examinations were unsuspicious in the vast majority of children (each >99%). Fever was present in one child. The socio-economic characteristics of rural and urban children differed greatly (Table 1).

One in four children was infected with an STH (positive microscopy or positive *Ascaris* PCR; Table 2), the prevalence was three times higher in rural (38%) than in urban children (13%). *A. lumbricoides* predominated largely, accounting for 95.6% of all detected helminths, whereas *T. trichiura* and hookworms were rare. Of the

**Table 1** Baseline characteristics of Rwandan schoolchildren, 2012

| Parameter | All | Stratum (school) | | $P$ |
|---|---|---|---|---|
| | | Gatovu (rural) | Ngoma (urban) | |
| Anthropometric and clinical parameters | | | | |
| No. | 622 | 301 | 321 | |
| Girls (%, $n$) | 52.3 (325/621) | 51.5 (155/301) | 53.1 (170/320) | 0.68 |
| Age (years; median, range; $n$ = 620) | 10.2 (4.9–17.9) | 10.9 (5.5–17.9) | 9.9 (4.9–15.9) | <0.0001 |
| Weight (kg; median, range; $n$ = 619) | 26.7 (13.8–64.4) | 26.2 (13.8–60.9) | 27.6 (15.5–64.4) | 0.16 |
| Height (cm; median, range; $n$ = 616) | 132 (103–168) | 130 (103–166) | 134 (104–168) | 0.008 |
| Weight-for-age Z (WAZ) score (median, range; children ≤10 years; $n$ = 304) | −0.9 (−4.0–3.1) | −1.3 (−4.0–0.9) | −0.5 (−2.9–3.1) | <0.0001 |
| WAZ<-2 (%, n; children ≤10 years) | 13.8 (42/304) | 23.3 (30/129) | 6.9 (12/175) | <0.0001 |
| Height-for-age Z (HAZ) score (median, range; $n$ = 613) | −1.2 (−6.1–4.2) | −1.8 (−6.1–1.5) | −0.6 (−5.4–4.2) | <0.0001 |
| HAZ<-2 (%, $n$) | 27.2 (167/613) | 43.3 (130/300) | 11.8 (37/313) | <0.0001 |
| Hb (g/dl; mean SD; $n$ = 616) | 13.3 ± 1.1 | 13.4 ± 1.2 | 13.2 ± 1.1 | 0.048 |
| Anaemia (%, $n$) | 18.2 (112/614) | 16.6 (49/295) | 19.7 (63/319) | 0.31 |
| Malaria test positive (%, $n$) | 2.6 (16/614) | 4.4 (13/295) | 0.9 (3/319) | 0.009 |
| Reduced appetite (%, $n$) | 3.3 (20/599) | 6.2 (18/292) | 0.7 (2/307) | 0.0001 |
| Any clinical abnormality (%, $n$) | 39.4 (244/619) | 49.2 (147/299) | 30.3 (97/320) | <0.0001 |
| Skin infection, fungal or bacterial (%, $n$) | 14.3 (89/619) | 19.4 (58/299) | 9.7 (31/320) | 0.0006 |
| Clinical malnutrition (%, $n$) | 9.4 (58/619) | 14.7 (44/299) | 4.4 (14/320) | <0.0001 |
| Lymph node swelling (%, $n$) | 5.7 (35/619) | 6.0 (18/299) | 5.3 (17/320) | 0.70 |
| Dental caries (%, $n$) | 9.4 (58/619) | 12.0 (36/299) | 6.9 (22/320) | 0.03 |
| Selected socio-demographic parameters | | | | |
| No. | 531 | 248 | 283 | |
| Mother, secondary or higher education (%, $n$) | 21.7 (115/530) | 8.9 (22/248) | 33.0 (93/282) | <0.0001 |
| Father, secondary or higher education (%, $n$) | 27.7 (146/527) | 9.0 (22/245) | 44.0 (124/282) | <0.0001 |
| Maternal occupation, farmer (%, $n$) | 54.8 (290/529) | 94.0 (233/248) | 20.3 (57/281) | <0.0001 |
| Paternal occupation, farmer (%, $n$) | 36.0 (191/531) | 67.3 (167/248) | 8.5 (24/283) | <0.0001 |
| Electricity at home (%, $n$) | 35.1 (180/513) | 0.4 (1/246) | 67.0 (179/267) | <0.0001 |
| Always able to pay health insurance (%, $n$) | 51.5 (272/528) | 30.6 (75/245) | 69.6 (197/283) | <0.0001 |
| Always able to pay school fees (%, $n$) | 53.8 (284/528) | 16.7 (41/245) | 85.9 (243/283) | <0.0001 |
| Animal ownership (%, $n$) | 50.3 (267/531) | 79.0 (196/248) | 25.1 (71/283) | <0.0001 |
| ≥2 household assets (%, $n$)* | 42.9 (228/531) | 22.2 (55/248) | 61.1 (173/283) | <0.0001 |
| Dump distance >100 m (%, $n$) | 19.8 (103/521) | 2.8 (7/247) | 35.0 (96/274) | <0.0001 |
| Garbage used as fertilizer (%, $n$) | 57.4 (301/524) | 86.7 (215/248) | 31.2 (86/276) | <0.0001 |
| In-house toilet facility (%, $n$) | 4.7 (25/531) | 1.2 (3/248) | 7.8 (22/283) | 0.0004 |
| Open water source (%, $n$) | 40.1 (213/531) | 77.0 (191/248) | 7.8 (22/283) | <0.0001 |
| Time to collect water <10 min (%, $n$) | 31.7 (153/482) | 0 (0/243) | 64.0 (153/239) | <0.0001 |

*Assessed household assets included lamp, radio, mobile phone, refrigerator, TV set.

151 *A. lumbricoides* infections, one-third was detected by PCR exclusively, and among the microscopically visible infections, two-thirds were of low intensity (Table 2). Setting *A. lumbricoides* detected by either microscopy or PCR as reference, the sensitivity of microscopy and PCR in diagnosing this helminth was 64.2% (95% CI, 66.0–71.8%) and 92.1% (95% CI, 86.2–95.6%), respectively (sensitivity, each 100% (95% CI, 99.0–100%)).

### Factors associated with STH infection

Interview data were available for 531 children. Many factors associated with STH infection in the total group reflected the differences in SES between rural and urban children (Table 1). Analysis was therefore performed separately for the rural and urban setting (Table 3). In rural children and in univariate analysis, absent maternal education and consumption of bitter leaf for helminth treatment were positively associated with STH infection, whereas increasing age, consumption of fruits and a long walking distance to collect water were negatively associated. In multivariate analysis among rural children, the odds of STH infection were increased for absent maternal education (aOR, 4.04; 95% CI, 1.8–9.2; $P$ = 0.0009) and consumption of bitter leaf (aOR, 3.52; 95% CI, 1.3–9.8; $P$ = 0.02), and reduced at higher age (9 < 12 years, aOR,

 

**Table 2** Pre-treatment parasitological parameters in 622 Rwandan schoolchildren

| Parameter | All | Stratum (school) | | P |
|---|---|---|---|---|
| | | Gatovu (rural) | Ngoma (urban) | |
| No. | 622 | 301 | 321 | |
| STH infection, all (%, *n*) | 25.4 (158) | 38.2 (115) | 13.4 (43) | <0.0001 |
| *Ascaris lumbricoides* (%, *n*) | 24.3 (151) | 37.5 (113) | 11.8 (38) | <0.0001 |
| Submicroscopic | 8.7 (54) | 11.6 (35) | 5.9 (19) | |
| Low | 12.1 (75) | 20.6 (62) | 4.1 (13) | |
| Moderate | 3.5 (22) | 5.3 (16) | 1.9 (6) | 0.07* |
| FEC (egp; geometric mean, 95% CI) | 1371 (999–1882) | 1245 (869–1782) | 2042 (1065–3916) | 0.23 |
| *Trichuris trichiura* (%, *n*) | 1.9 (12) | 2.3 (7) | 1.6 (5) | 0.49 |
| FEC (egp; geometric mean, 95% CI) | 72 (58–89) | 74 (55–98) | 69 (51–93) | 0.75 |
| Hookworm (%, *n*) | 0.3 (2) | 0.7 (2) | 0 | 0.23 |

Hookworm (both, 72 epg) and *Trichuris trichiura* infections were of low intensity.
*Global *P*-value for distribution of graded infection levels.

0.54; 95% CI, 0.3–1.1; $P = 0.08$; ≥12 years, aOR, 0.44, 95% CI, 0.2–0.9; $P = 0.02$) as well as in case of a walking distance of more than 1 h for water collection (aOR, 0.51; 95% CI, 0.3–0.9; $P = 0.02$).

In urban children, more factors were positively associated with STH infection than in rural children, including an open water source, bitter leaf consumption, ownership of pigs, goats, or any animal, a long walking distance to collect water, and use of garbage as fertilizer. Negatively associated factors were a higher than primary paternal education, a non-farming occupation of the mother, possession of more than two household assets, a distant dump site, electricity at home, attribution of STH infection to bare feet walking, awareness of using tap water for STH prevention, and meat consumption. In multivariate analysis of urban children, the odds of STH infection were increased with pig ownership (aOR, 8.04; 95% CI, 1.6–39.9; $P = 0.01$) and reduced for possession of more than two households assets (aOR, 0.16; 95% CI, 0.1–0.4; $P = 0.0002$), maternal occupation other than farming, trading or civil service (aOR, 0.24; 95% CI, 0.1–0.7; $P = 0.009$), and attribution of STH infection to bare feet walking (aOR, 0.22; 95% CI, 0.1–0.8; $P = 0.02$).

Neither in rural nor in urban children, STH infection was significantly associated with sex, hand washing practices (Table 3) or toilet facilities (data not shown).

### Manifestation of STH infection

STH infection was associated with reduced HAZ scores, stunting, any clinical finding, anaemia and low Hb levels only in urban children but not in their rural peers who exhibited already worse clinical parameters (Table 4). Clinically assessed malnutrition, in contrast, was associated with STH infection only in rural children.

Submicroscopic *A. lumbricoides* infection, in terms of clinical association, took an intermediate position between absent and microscopically visible infection (data not shown). Significant associations of submicroscopic infection were observed among urban children with increased proportions of stunting (26.3%, 5 of 19, $P = 0.04$) and anaemia (57.9%, 11 of 19, $P = 0.0001$), and reduced Hb concentrations (mean difference, −0.85 g/dl, $P = 0.01$).

### Effectiveness of preventive chemotherapy

Of the initial 622 children, stool samples were examined by microscopy and PCR for 510 (82.0%) individuals at 2 weeks after deworming. The cure rate on week 2 was 92% for any STH infection. Cure rates did not differ between helminth species, rural and urban children, with age, or with pre-intervention infection intensity (Table 5).

Among the eight non-cleared *A. lumbricoides* infections, seven were submicroscopic, and one had an FEC of 120 epg. Disregarding submicroscopic results both before and after treatment, the cure rate of *A. lumbricoides* infection was 100% (85 of 85).

### Reinfection rates

Of a total of 518 children presenting a stool sample 15 weeks after preventive chemotherapy, 364 initially uninfected or successfully treated children had their stool samples examined by both microscopy and PCR. These were included in the analysis of reinfection.

Overall, reinfection within 3 months occurred in 7.1% (26 of 364; *A. lumbricoides*, 5.8%, *T. trichiura*, 1.1%, hookworms, 0.5%). The reinfection rate was roughly three times higher in rural (10.7%, 20 of 187) than in

 

**Table 3** Prevalence of STH according to socio-economic and behavioural characteristics

| Parameter | Total | | | | Gatovu (rural) | | | | Ngoma (urban) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* |
| Sex | | | | | | | | | | | | |
| Female | 325 | 26.8 | 1 | | 155 | 34.9 | 1 | | 170 | 13.5 | 1 | |
| Male | 296 | 24.0 | 0.86 (0.6–1.3) | 0.43 | 146 | 41.3 | 0.76 (0.5–1.3) | 0.26 | 150 | 13.3 | 0.98 (0.5–2.0) | 0.96 |
| Age group (years) | | | | | | | | | | | | |
| <9 | 215 | 27.4 | 1 | | 86 | 48.8 | 1 | | 129 | 13.2 | 1 | |
| 9 <12 | 232 | 23.7 | 0.82 (0.5–1.3) | 0.37 | 107 | 36.4 | 0.60 (0.3–1.1) | 0.08 | 125 | 12.8 | 0.97 (0.4–2.1) | 0.93 |
| ≥12 | 173 | 24.9 | 0.87 (0.5–1.4) | 0.57 | 107 | 30.8 | 0.47 (0.3–0.9) | 0.01 | 66 | 15.2 | 1.18 (0.5–2.9) | 0.71 |
| Maternal education | | | | | | | | | | | | |
| Primary | 342 | 26.3 | 1 | | 192 | 33.3 | 1 | | 150 | 17.3 | 1 | |
| None | 73 | 38.4 | 1.74 (1.0–3.1) | 0.04 | 34 | 61.8 | 3.23 (1.4–7.4) | 0.002 | 39 | 17.9 | 1.04 (0.4–2.8) | 0.93 |
| Else | 115 | 10.4 | 0.33 (0.2–0.6) | 0.0004 | 22 | 18.2 | 0.44 (0.1–1.4) | 0.15 | 93 | 8.6 | 0.45 (0.2–1.1) | 0.06 |
| Paternal education | | | | | | | | | | | | |
| Primary | 267 | 28.8 | 1 | | 150 | 33.3 | 1 | | 117 | 23.1 | 1 | |
| None | 114 | 34.2 | 1.28 (0.8–2.1) | 0.30 | 73 | 42.5 | 1.48 (0.8–2.7) | 0.18 | 41 | 19.5 | 0.81 (0.3–2.1) | 0.64 |
| Else | 146 | 8.2 | 0.22 (0.1–0.4) | <0.0001 | 22 | 27.3 | 0.75 (0.2–2.2) | 0.57 | 124 | 4.8 | 0.17 (0.1–0.5) | <0.0001 |
| Maternal occupation | | | | | | | | | | | | |
| Farmer | 290 | 35.5 | 1 | | 233 | 36.5 | 1 | | 57 | 31.6 | 1 | |
| Trader | 86 | 16.3 | 0.35 (0.2–0.7) | 0.0007 | 0 | – | – | | 86 | 16.3 | 0.42 (0.2–1.0) | 0.03 |
| Civil servant | 48 | 10.4 | 0.21 (0.1–0.6) | 0.0006 | 10 | 20.0 | 0.44 (0.0–2.3) | 0.50 | 38 | 7.9 | 0.19 (0.0–0.7) | 0.006 |
| Else | 105 | 7.6 | 0.15 (0.1–0.3) | <0.0001 | 5 | 40.0 | 1.16 (0.1–10.3) | 1.0 | 100 | 6.0 | 0.14 (0.0–0.4) | <0.0001 |
| Paternal occupation | | | | | | | | | | | | |
| Farmer | 191 | 33.5 | 1 | | 167 | 34.7 | 1 | | 24 | 25.0 | 1 | |
| Trader | 30 | 20.0 | 0.50 (0.2–1.3) | 0.14 | 0 | – | – | | 30 | 20.0 | 0.75 (0.2–3.2) | 0.66 |
| Civil servant | 85 | 10.6 | 0.23 (0.1–0.5) | <0.0001 | 7 | 28.6 | 0.75 (0.1–4.8) | 1.0 | 78 | 9.0 | 0.30 (0.1–1.2) | 0.07 |
| Else | 122 | 17.2 | 0.41 (0.2–0.8) | 0.002 | 7 | 42.9 | 1.41 (0.2–8.6) | 0.70 | 115 | 15.7 | 0.56 (0.2–2.0) | 0.37 |
| Absent/died | 103 | 29.1 | 0.82 (0.5–1.4) | 0.44 | 67 | 38.8 | 1.19 (0.6–2.2) | 0.56 | 36 | 11.1 | 0.38 (0.1–1.9) | 0.18 |
| Able to pay health insurance fees (%, *n*) | | | | | | | | | | | | |
| Always | 272 | 18.0 | 1 | | 75 | 28.0 | 1 | | 197 | 14.2 | 1 | |
| Not always | 256 | 30.5 | 1.99 (1.3–3.1) | 0.0008 | 170 | 38.2 | 1.59 (0.9–3.0) | 0.12 | 86 | 15.1 | 1.07 (0.5–2.3) | 0.84 |
| Able to pay school fees (%, *n*) | | | | | | | | | | | | |
| Always | 284 | 18.3 | 1 | | 41 | 36.6 | 1 | | 243 | 15.2 | 1 | |
| Not always | 244 | 31.1 | 2.02 (1.3–3.9) | 0.0006 | 204 | 35.3 | 0.95 (0.5–2.0) | 0.87 | 40 | 10.0 | 0.62 (0.2–1.9) | 0.38 |
| Household assets | | | | | | | | | | | | |
| None | 166 | 36.1 | 1 | | 103 | 39.8 | 1 | | 63 | 30.2 | 1 | |
| One | 137 | 25.5 | 0.61 (0.4–1.0) | 0.05 | 90 | 28.9 | 0.61 (0.3–1.2) | 0.11 | 47 | 19.1 | 0.55 (0.2–1.5) | 0.19 |
| Two or more | 228 | 15.4 | 0.32 (0.2–0.5) | <0.0001 | 55 | 40.0 | 1.01 (0.5–2.1) | 0.98 | 173 | 7.5 | 0.19 (0.1–0.4) | <0.0001 |

**Table 3** (*Continued*)

| | Total | | | | Gatovu (rural) | | | | Ngoma (urban) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* |
| Electricity at home | | | | | | | | | | | | |
| Absent | 333 | 33.3 | 1 | | 245 | 35.5 | 1 | | 88 | 27.3 | 1 | |
| Present | 180 | 7.8 | 0.17 (0.1–0.3) | <0.0001 | 1 | 0 | 0.0 (0.0–71.3) | 1.0 | 179 | 7.8 | 0.23 (0.1–0.5) | <0.0001 |
| Animal ownership | | | | | | | | | | | | |
| No | 264 | 15.5 | 1 | | 52 | 36.5 | 1 | | 212 | 10.4 | 1 | |
| Yes | 267 | 33.3 | 2.72 (1.8–4.2) | <0.0001 | 196 | 35.7 | 0.96 (0.5–1.9) | 0.91 | 71 | 26.8 | 3.16 (1.5–6.6) | 0.0007 |
| Goat ownership | | | | | | | | | | | | |
| No | 384 | 20.6 | 1 | | 144 | 36.1 | 1 | | 240 | 11.3 | 1 | |
| Yes | 147 | 34.7 | 2.05 (1.3–3.2) | 0.0007 | 104 | 35.6 | 0.98 (0.6–1.7) | 0.93 | 43 | 32.6 | 3.81 (1.7–8.6) | 0.0003 |
| Pig ownership | | | | | | | | | | | | |
| No | 389 | 19.8 | 1 | | 115 | 34.8 | 1 | | 274 | 13.5 | 1 | |
| Yes | 142 | 37.3 | 2.41 (1.6–3.8) | <0.0001 | 133 | 36.8 | 1.09 (0.6–1.9) | 0.74 | 9 | 44.4 | 5.12 (1.0–24.8) | 0.03 |
| Dump, distance | | | | | | | | | | | | |
| <5 m | 144 | 33.3 | 1 | | 97 | 37.1 | 1 | | 47 | 25.5 | 1 | |
| 5–100 m | 274 | 25.9 | 0.70 (0.4–1.1) | 0.11 | 143 | 34.3 | 0.88 (0.5–1.6) | 0.65 | 131 | 16.8 | 0.59 (0.3–1.4) | 0.19 |
| >100 m | 103 | 8.7 | 0.19 (0.1–0.4) | <0.0001 | 7 | 42.9 | 1.27 (0.2–8.0) | 1.0 | 96 | 6.3 | 0.19 (0.1–0.6) | 0.001 |
| Garbage use | | | | | | | | | | | | |
| Disposed/burned/else | 223 | 14.8 | 1 | | 33 | 33.3 | 1 | | 190 | 11.6 | 1 | |
| Fertilizer | 301 | 32.2 | 2.74 (1.7–4.4) | <0.0001 | 215 | 36.3 | 1.14 (0.5–2.7) | 0.74 | 86 | 22.1 | 2.17 (1.1–4.5) | 0.02 |
| Water source | | | | | | | | | | | | |
| Own tap | 196 | 10.2 | 1 | | 0 | – | – | | 196 | 10.2 | 1 | |
| Shared tap | 122 | 27.0 | 3.26 (1.7–6.3) | <0.0001 | 57 | 38.6 | 1 | | 65 | 16.9 | 1.79 (0.8–4.2) | 0.15 |
| Open source | 213 | 36.2 | 4.98 (2.8–8.9) | <0.0001 | 191 | 35.1 | 0.86 (0.5–1.7) | 0.62 | 22 | 45.5 | 7.33 (2.5–21.2) | 0.0001 |
| Time needed to collect water (%, *n*) | | | | | | | | | | | | |
| <5 min | 86 | 9.3 | 1 | | 0 | – | – | | 86 | 9.3 | 1 | |
| 5 <10 min | 67 | 17.9 | 2.13 (0.8–6.2) | 0.12 | 0 | – | – | | 67 | 17.9 | 2.13 (0.8–6.2) | 0.12 |
| 10 <60 min | 196 | 35.2 | 5.30 (2.3–12.6) | <0.0001 | 110 | 45.6 | 1 | | 86 | 22.1 | 2.76 (1.1–7.4) | 0.02 |
| ≥60 min | 133 | 28.6 | 3.90 (1.6–9.7) | 0.0006 | 133 | 28.6 | 0.48 (0.3–0.8) | 0.006 | 0 | – | – | – |
| Walking distance to nearest health centre | | | | | | | | | | | | |
| ≤15 min | 136 | 13.2 | 1 | | 1 | 0 | 0.0 (0.0–75.6) | 1.0 | 135 | 13.3 | 1 | |
| 15–60 min | 184 | 20.7 | 1.71 (0.9–3.3) | 0.08 | 46 | 34.8 | 1 | | 138 | 15.9 | 1.23 (0.6–2.6) | 0.54 |
| >60 min | 200 | 36.5 | 3.77 (2.1–7.0) | <0.0001 | 199 | 36.7 | 1.09 (0.5–2.3) | 0.81 | 1 | 0 | 0.0 (0.0–255) | 1.0 |
| Meat consumption | | | | | | | | | | | | |
| No | 289 | 34.6 | 1 | | 226 | 37.6 | 1 | | 63 | 23.8 | 1 | |
| Yes | 233 | 12.1 | 0.26 (0.2–0.4) | <0.0001 | 15 | 26.7 | 0.60 (0.1–2.1) | 0.40 | 218 | 11.0 | 0.40 (0.2–0.9) | 0.01 |
| Fruit consumption | | | | | | | | | | | | |
| No | 147 | 24.5 | 1 | | 57 | 49.1 | 1 | | 90 | 8.9 | 1 | |
| Yes | 380 | 24.5 | 1.0 (0.6–1.6) | 1.0 | 189 | 32.3 | 0.49 (0.3–0.9) | 0.02 | 191 | 16.8 | 2.06 (0.9–5.1) | 0.08 |

**Table 3** (*Continued*)

| Parameter | Total | | | | Gatovu (rural) | | | | Ngoma (urban) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* | No. | % STH infected | OR (95%CI) | *P* |
| Hand washing before eating | | | | | | | | | | | | |
| No | 48 | 37.5 | 1 | | 35 | 48.6 | 1 | | 13 | 7.7 | 1 | |
| Yes | 473 | 23.3 | 0.51 (0.3–1.0) | 0.03 | 210 | 33.8 | 0.54 (0.3–1.2) | 0.09 | 263 | 14.8 | 2.09 (0.3–91.6) | 0.70 |
| Hand washing after toilet use | | | | | | | | | | | | |
| No | 167 | 32.9 | 1 | | 155 | 35.5 | 1 | | 12 | 0 | 1 | |
| Yes | 352 | 20.6 | 0.53 (0.3–0.8) | 0.002 | 90 | 36.7 | 1.05 (0.6–1.9) | 0.85 | 264 | 15.2 | ud (ud–ud) | 0.23 |
| Hand washing after playing | | | | | | | | | | | | |
| No | 436 | 27.8 | 1 | | 243 | 36.2 | 1 | | 193 | 17.1 | 1 | |
| Yes | 85 | 8.2 | 0.23 (0.1–0.5) | 0.0001 | 2 | 0 | 0.0 (0.0–9.5) | 0.54 | 83 | 8.4 | 0.45 (0.2–1.1) | 0.06 |
| Awareness of STH prevention using tap water | | | | | | | | | | | | |
| No | 355 | 27.3 | 1 | | 155 | 40.0 | 1 | | 200 | 17.5 | 1 | |
| Yes | 168 | 19.0 | 0.63 (0.4–1.0) | 0.04 | 92 | 29.3 | 0.62 (0.4–1.1) | 0.09 | 76 | 6.6 | 0.33 (0.1–0.9) | 0.02 |
| Awareness of STH prevention by cooking water | | | | | | | | | | | | |
| No | 30 | 40.0 | 1 | | 29 | 41.4 | 1 | | 1 | 0 | 1 | |
| Yes | 493 | 23.7 | 0.47 (0.2–1.1) | 0.04 | 218 | 35.3 | 0.77 (0.3–1.8) | 0.52 | 275 | 14.5 | ud (ud–ud) | 1.0 |
| Bitter leaf commonly taken for treatment of helminths | | | | | | | | | | | | |
| No | 496 | 22.8 | 1 | | 224 | 33.5 | 1 | | 272 | 14.0 | 1 | |
| Yes | 26 | 57.7 | 4.62 (1.9–11.1) | <0.0001 | 20 | 60.0 | 2.98 (1.1–8.4) | 0.02 | 6 | 50.0 | 6.16 (0.8–47.2) | 0.04 |
| Attribution of helminths infection to shaking hands | | | | | | | | | | | | |
| No | 434 | 22.4 | 1 | | 163 | 36.2 | 1 | | 271 | 14.0 | 1 | |
| Yes | 89 | 36.0 | 1.95 (1.2–3.3) | 0.007 | 84 | 35.7 | 0.98 (0.5–1.8) | 0.94 | 5 | 40.0 | 4.09 (0.3–36.3) | 0.15 |
| Attribution of helminth infection to bare feet walking | | | | | | | | | | | | |
| No | 148 | 38.5 | 1 | | 130 | 38.5 | 1 | | 18 | 38.9 | 1 | |
| Yes | 375 | 19.2 | 0.38 (0.2–0.6) | <0.0001 | 117 | 33.3 | 0.80 (0.5–1.4) | 0.40 | 258 | 12.8 | 0.23 (0.1–0.7) | 0.008 |

ud, undefined.

**Table 4** Clinical characteristics of Rwandan schoolchildren separated by STH infection and rural *vs.* urban setting

| | Gatovu (rural) | | | Ngoma (urban) | | |
|---|---|---|---|---|---|---|
| Parameter | Non-infected | Infected | *P* | Non-infected | Infected | *P* |
| No. | 186 | 115 | | 278 | 43 | |
| Age (median, range) | 11.2 (5.5–17.9) | 10.0 (5.8–17.0) | 0.02 | 9.9 (4.9–15.4) | 10.0 (5.2–15.9) | 0.82 |
| Hb (g/dl; mean ± SD) | 13.4 ± 1.2 | 13.3 ± 1.0 | 0.31 | 13.2 ± 1.1 | 12.8 ± 1.3 | 0.03 |
| Anaemia (%, *n*) | 17.9 (33/184) | 14.4 (16/111) | 0.43 | 17.0 (47/277) | 38.1 (16/42) | 0.001† |
| WAZ score (median, range; ≤10 years) | −1.26 (−4.05–0.60) | −1.27 (−3.16–0.91) | 0.45 | −0.45 (−2.87–3.12) | −0.91 (−2.62–2.01) | 0.13 |
| Underweight (≤10 years; %, *n*) | 21.4 (15/70) | 25.4 (15/59) | 0.59 | 5.9 (9/153) | 13.6 (3/22) | 0.18 |
| HAZ score (median, range) | −1.86 (−5.51–1.51) | −1.79 (−6.10–1.41) | 0.59 | −0.49 (−5.43–4.22) | −1.18 (−4.11–3.28) | 0.003 |
| Stunting (%, *n*) | 44.6 (83/186) | 41.2 (47/114) | 0.56 | 9.6 (26/270) | 25.6 (11/43) | 0.003‡ |
| Any clinical abnormality (%, *n*) | 50.3 (93/185) | 47.4 (54/114) | 0.63 | 28.2 (78/277) | 44.2 (19/43) | 0.03§ |
| Clinical malnutrition (%, *n*) | 11.4 (21/185) | 20.2 (23/114) | 0.04* | 4.3 (12/277) | 4.7 (2/43) | 1.0 |

Adjusted odds ratios (95% confidence intervals) and [covariates] from logistic regression models with backward removal: *1.65 (0.83–3.26) [age]; †3.38 (1.52–7.51) [household assets, distance to dump site]; ‡2.41 (0.98–5.95) [age, meat consumption], §2.04 (1.05–3.96) [age].

**Table 5** Cure rates of mebendazole against soil-transmitted helminths in Rwandan children

| | Cure rate (%, *n/n*) |
|---|---|
| All | 92.4 (122/132) |
| Species | |
| *Ascaris lumbricoides* | 93.7 (118/126) |
| *Trichuris trichiura* | 100 (11/11) |
| Hookworms | 100 (2/2) |
| School | |
| Rural | 91.8 (89/97) |
| Urban | 94.3 (33/35) |
| Age group (years) | |
| 5 <9 | 91.1 (41/45) |
| 9 <12 | 93.6 (44/47) |
| ≥12 | 92.3 (36/39) |
| Infection intensity | |
| Submicroscopic | 95.7 (44/46) |
| Low | 89.9 (62/69) |
| Moderate | 94.1 (16/17) |

urban children (3.4%, 6 of 177; $P = 0.007$). Of the reinfections, 30.8% were submicroscopic, and 57.7% and 11.5% were of low and moderate intensity, respectively. The geometric mean epg count was 640 (95% CI, 246–1667) for *A. lumbricoides* and 102 (95% CI, 61–170) for *T. trichiura*.

None of the factors influencing STH infection at recruitment (Table 3) was significantly associated with reinfection among rural children. Among urban children, reinfection was negatively associated with meat consumption (OR, 0.07 (95% CI, 0.0–0.01), $P = 0.01$) and attribution of STH infection to bare feet walking (OR, 0.10 (95% CI, 0.01–1.35); $P = 0.04$). Notably, the odds of reinfection were greatly increased in initially infected children (rural, OR, 2.56; 95% CI, 0.92–7.20; $P = 0.04$; urban, OR, 24.31; 95% CI, 3.02–279.8; $P = 0.0008$), and even more at moderate infection intensity (Table 6). Age and sex had no influence on reinfection, and in multivariate analysis including some univariately associated SES parameters ($P < 0.10$; data not shown), the initial infection status remained the only significant predictor of reinfection.

## Discussion

Five years after the implementation of a nationwide STH control programme in Rwanda (Ruxin & Negin 2012), the present study among schoolchildren from the southern highlands showed a low to moderate prevalence of STH infection, predominantly with *A. lumbricoides* (rural, 38%; urban, 13%). Rural–urban differences in the determinants of STH infection were obvious, although they overall included factors linked to poverty, education, sanitation, water supply and behaviour. No evidence for a diminished effectiveness of mebendazole was seen.

In comparison with the STH prevalence in 2008 among schoolchildren in Huye district of 72% (Center for

 

**Table 6** Reinfection rates of STHs in 364 Rwandan children following mebendazole treatment

| | Reinfection rates of STHs at 15 weeks post-treatment (%) | | |
|---|---|---|---|
| | Total | Rural school | Urban school |
| Age (years) | | | |
| 5 >9 | 6.5 (7/107) | 13.6 (6/44) | 1.6 (1/63) |
| 9 <12 | 5.0 (7/139) | 7.4 (5/68) | 2.8 (2/71) |
| ≥12 | 10.3 (12/117) | 12.0 (9/75) | 7.1 (3/42) |
| Sex | | | |
| Female | 5.9 (11/186) | 10.4 (10/96) | 1.1 (1/90) |
| Male | 8.5 (15/177) | 11.0 (10/91) | 5.8 (5/86) |
| Pre-treatment infection status | | | |
| Non-infected | 3.9 (11/282) | 7.4 (9/122) | 1.3 (2/160) |
| Infected | 18.3 (15/82)* | 16.9 (11/65)* | 23.5 (4/17)* |
| Pre-treatment infection intensity | | | |
| None | 3.9 (11/282) | 7.4 (9/122) | 1.3 (2/160) |
| Submicroscopic | 4.5 (1/22) | 0 (0/17) | 20.0 (1/5) |
| Low | 19.6 (9/46) | 18.9 (7/37) | 22.2 (2/9) |
| Moderate | 35.7 (5/14)* | 36.4 (4/11)* | 33.3 (1/3)* |

*Significant association, $P < 0.05$.

Infectious Disease Control Rwanda 2008), the present figure indicates a substantial decline. Even though our study sample is not representative for Huye district, let alone Rwanda, because children from only two schools were examined, this decline likely illustrates the magnitude of impact of stringently implemented preventive chemotherapy. At the same time, the remaining STH burden points to the need for additional interventions as to water, sanitation and hygiene (WASH) for efficient and sustainable helminth control. Based on Rwanda Demographic and Health Survey data (National Institute of Statistics of Rwanda 2012), some 25% of the population in 2010 relied on unimproved sources of drinking water (e.g. unprotected springs) considered unhealthy. The most common source (38%) was protected spring water, followed by public tap (26%). Half of the households did not treat their water prior to drinking. Only 1.1% of the population had an individual flush toilet; the vast majority used pit latrines. In the present study, 40% of respondents relied on open water sources, 5% had an in-house toilet and these figures were even less favourable for rural dwellers. Remarkably, more individual factors associated with STH infection could be identified among urban schoolchildren. This may reflect a higher heterogeneity among urban dwellers, and *vice versa*, a rather homogenous, generally poor, agricultural, rural population. Likewise, in rural villages in the study area, socio-economic factors did not play a major role in predicting another intestinal parasite, *Giardia duodenalis* (Ignatius *et al.* 2012). Parental education, issues of water collection and consumption of bitter leaf were associated factors, overlapping between rural and urban schoolchildren. While the first two are not surprising, an increased risk of STH infection in children whose assumed helminth infection was commonly treated with bitter leaf is remarkable. In fact, bitter leaf (*Vernonia amygdalina*) has shown anthelminthic activity *in vitro* (Ademola & Eloff 2011). Possibly, the positive association of STH infection and bitter leaf reflects parental notice of the former and incomplete or insufficient home treatment with the latter. Pig ownership greatly increased the odds of STH infection among urban dwellers. Although based on small numbers, this finding lends support to the notion that *A. suum* may act as a source of human ascariasis considering that *A. suum* and *A. lumbricoides* are at least closely related species (Cavallero *et al.* 2013). Also in this regard, our preliminary data suggest that the PCR assay used in the present study yields a positive result in the presence of *A. suum*. Hand washing practises showed only weak associations with STH infection. Still, the low proportion of particularly rural children washing hands after toilet use or playing indicates that improved hygiene may be rewarding. The same applies to an easy availability of safe water. The impact of WASH interventions and health education on STHs is unquestioned (Esrey *et al.* 1991; Asaolu & Ofoezie 2003; Ziegelbauer *et al.* 2012). Thus, there is an urgent need for intersectoral integration of WASH activities into treatment-based STH control programmes, and to overcome respective barriers (Freeman *et al.* 2013a).

STH infection was associated with stunting and anaemia in urban children only. This was also seen for submicroscopic infection suggesting their clinical relevance. In rural children who showed more than three times higher rates of underweight and stunting than their urban peers, an impact of predominantly low-level STH infections on stunting might have been covered by more direct contributors, for example underfeeding. Malnutrition is a well-known consequence of STH infection (reviewed by Bethony *et al.* 2006). In the present study, adjustment for age and/or proxy indicators of socio-economic status weakened the association with malnutrition. This does not necessarily argue against a causative role of the STHs but rather indicates the multifactorial aetiology of malnutrition and the overlap of its risk factors with those for helminth infection. Although made up of almost entirely *A. lumbricoides* while hookworm was rare, STH infection increased the odds of anaemia in urban children. *A. lumbricoides* infection can cause decreased appetite and nutrient intake as well as malabsorption of vitamin A and possibly other nutrients (reviewed by Bethony *et al.* 2006), but evidence for a role in anaemia is ambiguous (Ngui *et al.* 2012; Abanyie *et al.* 2013; Suchdev *et al.* 2014). Vitamin A deficiency secondary to *Ascaris* infection may potentially contribute to anaemia (Al-Mekhlafi *et al.* 2013), but we currently have no data to substantiate this hypothesis.

To our knowledge, this is the first study applying PCR-based diagnosis of *A. lumbricoides* to preventive chemotherapy. PCR assays have previously shown superior sensitivity in the detection of STHs (Basuni *et al.* 2011; Jonker *et al.* 2012; van Mens *et al.* 2013). In the present study, one-third of the initial *A. lumbricoides* infections and of those detected after 3 months were diagnosed by PCR exclusively as were seven of the eight infections 2 weeks following treatment. Excluding submicroscopic infections from the study would raise the overall cure rate from 92% to 100%. This may indicate that cure rates based on microscopy are overestimations, but more data are needed for firm conclusions. Also, as a limitation of the present study, microscopic STH diagnosis was based on single Kato-Katz smears. While this is common practice when resources are limited, light infections may have been missed because of poor sensitivity and fluctuations in egg excretion (de Vlas & Gryseels 1992; Engels *et al.* 1996). In this regard, the diagnostic superiority of PCR assays needs to be confirmed applying more sensitive microscopic tools, for example duplicate Kato-Katz smears or FLOTAC, and in areas of differing endemicity and distribution of helminth species (Knopp *et al.* 2014). So far, PCR methods may help to improve the accuracy of STH diagnosis and the early detection of low-grade infections persisting after treatment, for example due to resistance. Whether submicroscopic infections form a so far unrecognized reservoir of transmission will be a relevant aspect of future investigations. However, considering the operational requirements and costs associated with PCR assays, the widespread use of this technique in endemic regions and beyond research settings is unlikely.

The present study revealed a very high effectiveness of mebendazole. The high cure rate against *A. lumbricoides* accords with data from meta-analysis (95%) but contrasts with figures estimated for *T. trichiura* (36%) and hookworm (15%) (Keiser & Utzinger 2008). Recent data suggest *A. lumbricoides* to be comparatively robust against the drug-induced selection of resistance mutations (Diawara *et al.* 2013). Notwithstanding the molecular causes, our findings of high effectiveness are reassuring considering the intense drug pressure on human STHs in Rwanda in recent years (Ruxin & Negin 2012). Still, as a limitation, our results stem from an observational study and not a treatment trial, and more geographical coverage is needed to provide a picture on the actual effectiveness of preventive chemotherapy in Rwanda.

Reinfection within 3 months occurred in 7% which is below the overall figure of 26% for *A. lumbricoides* estimated in a recent meta-analysis (Jia *et al.* 2012). Pre-treatment prevalence and intensity, that is transmission intensity, are the main determinants of reinfection, which accords with findings of the present study. Further determinants were virtually absent in the present study which may also be due to the comparatively low sample size and prevalence of infection at the three-month follow-up visit. Nevertheless, one quarter of initially infected rural children was reinfected within 3 months. While the overall aim and success of preventive chemotherapy is reduced morbidity but not necessarily the absence of helminths (Bethony *et al.* 2006; World Health Organisation 2006), further efforts are thus needed to reduce transmission. In this regard, the impact of school-based deworming on community transmission is unclear. In low-income settings, some 20–30% of the population are of school age, and for *A. lumbricoides* and *T. trichiura* (but not hookworms), the highest intensity infections are in this age group. Thus, school-based deworming may target up to 50% of all *A. lumbricoides* in a community which may contribute to a reduced overall intensity of transmission. Recent modelling estimates, however, suggest that this impact may be limited (Anderson *et al.* 2013). This supports the inclusion of other target groups for deworming, for example pre-school children and adults (World Health Organisation 2006), but also that additional interventions are needed for long-term STH control. For instance, in a recent study in Kenya, a school-based helminth control programme including hygiene promotion, water treatment technology and sanitation infrastructure could almost halve the odds of reinfection following deworming (Freeman *et al.* 2013b).

In conclusion, STH infections are prevalent and associated with morbidity among schoolchildren in this area of southern Rwanda. The multifaceted pattern of associated factors points to the need and opportunity of targeted interventions aiming at reducing transmission. School-based preventive chemotherapy is highly effective in this area, but more data from other geographical regions of the country as well as continuous monitoring of effectiveness are needed.

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**Corresponding Author Frank P. Mockenhaupt**, Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Spandauer Damm 130, 14050 Berlin, Germany. Tel.: +49 30 30116 815; Fax: +49 30 30116 888; E-mail: frank.mockenhaupt@charite.de

# Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

# Vollständige Publikationsliste von Olga Staudacher

1. Soil-transmitted helminths in southern highland Rwanda: associated factors and effectiveness of school-based preventive chemotherapy.
Staudacher O, Heimer J, Steiner F, Kayonga Y, Havugimana JM, Ignatius R, Musemakweri A, Ngabo F, Harms G, Gahutu JB, Mockenhaupt FP.
Trop Med Int Health. 2014 Jul;19(7):812-24.

2. Age-dependent decline and association with stunting of Giardia duodenalis infection among schoolchildren in rural Huye district, Rwanda.
Heimer J, Staudacher O, Steiner F, Kayonga Y, Havugimana JM, Musemakweri A, Harms G, Gahutu JB, Mockenhaupt FP.
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