Acta Tropica 171 (2017) 186–193

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Epidemiology of polyparasitism with *Taenia solium*, schistosomes and soil-transmitted helminths in the co-endemic village of Malanga, Democratic Republic of Congo

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ARTICLE INFO

Keywords:
Polyparasitism
Co-infection
Helminths
Taenia solium
Schistosoma
Soil-transmitted helminths
Democratic Republic of Congo

ABSTRACT

Helminth co-infections are common in sub-Saharan Africa. However, little is known about the distribution and determinants of co-infections with *Taenia solium* taeniasis/cysticercosis. Building on a previous community-based study on human cysticercosis in Malanga village, we investigated co-infections with *Taenia solium*, soil-transmitted helminths (STHs) and *Schistosoma* spp and associated risk factors in a random subsample of 330 participants. Real time PCR assays were used to detect DNA of soil-transmitted helminths (STHs), *T. solium* and *Schistosoma* in stool samples and *Schistosoma* DNA in urine samples. Serum samples were tested for *T. solium* cysticercosis using the B158/B60 monoclonal antibody-based antigen ELISA. Bivariate analysis and logistic regression were applied to assess associations of single and co-infections with common risk factors (age, sex, area, hygiene) as well as pair wise associations between helminth species.

Overall, 240 (72.7%) participants were infected with at least one helminth species; 128 (38.8%) harbored at least two helminth species (16.1% with STHs-*Schistosoma*, 14.5% with STHs-*T. solium* taeniasis/cysticercosis and 8.2% with *Schistosoma*-*T. solium* taeniasis/cysticercosis co-infections). No significant associations were found between *Schistosoma*-*T. solium* taeniasis/cysticercosis co-infection and any of the risk factors studied. Males (OR = 2 (95%CI = 1.1–5), p = 0.03) and open defecation behavior (OR = 3.8 (95%CI = 1.1–6.5), p = 0.04) were associated with higher odds of STHs-*T. solium* taeniasis/cysticercosis co-infection. Village districts that were found at high risk of *T. solium* taeniasis/cysticercosis were also at high risk of co-infection with STHs and *T. solium* taeniasis/cysticercosis (OR = 3.2 (95%CI = 1.1–7.8), p = 0.03). Significant pair-wise associations were found between *T. solium* cysticerci and *Necator americanus* (OR = 2.2 (95%CI = 1.2–3.8), p < 0.01) as well as *Strongyloides stercoralis* (OR = 2.7 (95%CI = 1.1–6.5), p = 0.02).

These findings show that co-infections with *T. solium* are common in this polyparasitic community in DRC. Our results on risk factors of helminth co-infections and specific associations between helminths may contribute to a better integration of control within programmes that target more than one NTD.

1. Introduction

Schistosomiasis, soil-transmitted helminths (STHs) and *Taenia solium* infections are part of the common neglected tropical diseases (NTDs) in rural and poor urban settings of sub-Saharan Africa (Hotez and Kamath, 2009). STHs and *Schistosoma* spp can cause long-term chronic morbidity including anemia and iron-deficiency (Friedman et al., 2005; Hall et al., 2008; Jonker et al., 2012), malnutrition and impaired child growth and cognition (Jukes et al., 2002; Stephenson and Latham, 2000). In addition, *Schistosoma* infections may cause liver

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http://dx.doi.org/10.1016/j.actatropica.2017.03.019
Received 16 September 2015; Received in revised form 10 March 2017; Accepted 18 March 2017
Available online 20 March 2017
0001-706X/

and urogenital tissue damage (Gryseels et al., 2006). *T. solium* infections include taeniasis (infection with theadult tapeworm following consumption of under-cooked or raw pig meat) and cysticercosis (infection with the larval stage of the parasite after ingestion of eggs with fecally-contaminated water or food). *T. solium*-related morbidity is strongly associated with neurocysticercosis, a major cause of epilepsy and various neurological disorders within endemic regions (Garcia et al., 2003; Ndimubanzi et al., 2010). Geographic distributions of these parasitic worms overlap in many endemic territories. Moreover, some species share transmission routes, risk factors, risk groups and/or within-host habitats. Consequently, concomitant infections with multiple parasite species can occur in the human host, with potential interactions. Knowledge about the distribution and determinants of helminth co-infections are useful for designing integrated control programs that target multiple infections at once (Brooker et al., 2009).

Over the two past decades, increased attention has been given to polyparasitism with helminths (schistosomes and STHs) as well as to helminth-plasmodium co-infections. These studies have shown that school-age children are at the highest risk of co-infections and that polyparasitism is associated with higher odds of morbidity than single infections (for a review see (Brooker et al., 2007; Naing et al., 2013; Supali et al., 2010; Vaumourin et al., 2015; Viney and Graham, 2013)). Moreover, co-infections seem to be more common in children (mainly boys) from households with poor socioeconomic status (Bisanzio et al., 2014; Raso et al., 2006) and low access to sanitation and clean water (Bisanzio et al., 2014; Righetti et al., 2012). The geo-spatial distribution of helminth co-infections is markedly heterogeneous at both local and regional scales, depending on individual (genetic, sex, age), household (socioeconomic status, crowding), geographical (elevation, distance to water bodies) and climatic (temperature) factors (Raso et al., 2006; Brooker and Clements, 2009; Ellis et al., 2007; Pullan et al., 2008; Soares Magalhaes et al., 2011). The distribution of co-infections with STHs and *Schistosoma* as well as helminths-*Plasmodium* co-infection has been found to be determined by the distribution of the least common species and its environmental risk factors (Brooker and Clements, 2009; Soares Magalhaes et al., 2011; Brooker et al., 2006; Brooker et al., 2012; Yajima et al., 2011).

Despite this growing interest in helminth co-infections, little is known about the epidemiology of co-infections with *T. solium* taeniasis/cysticercosis. A recent review on *T. solium* taeniosis/cysticercosis and the co-distribution with schistosomiasis in Africa mentioned the scarcity of such data, due to under-reporting of *T. solium* infections in many African countries (Braae et al., 2015). One of the reasons is the lack of a good field-applicable diagnostic tool enabling simultaneous detection of STHs, *Schistosoma* spp and *Taenia* spp. Indeed, schistosomiasis and STHs infections are commonly diagnosed using coprology, which is neither sensitive enough for detecting *Taenia* spp nor specific for distinguishing *T. solium* and *T. saginata* eggs (Praet et al., 2013). Recent development of DNA-based methods has enabled discrimination of morphologically identical species, and combination of PCR assays has allowed the simultaneous detection of different intestinal parasite species (Gordon et al., 2011). This provides an interesting opportunity to study the epidemiology of polyparasitism for a broader number of parasite species.

The Democratic Republic of Congo (DRC) is estimated to have the second or third highest number of cases of schistosomiasis and STHs infections in sub-Saharan Africa (Hotez and Kamath, 2009) but there is an overall paucity of data supporting this statement (Rimoin and Hotez, 2013). The country is also reported to be endemic for *T. solium*, though data are scarce (Zoli et al., 2003). Recent surveys conducted in some villages of Kimpese health district (in Bas-Congo province) point to co-endemicity of *Schistosoma*, STHs and *T. solium* infections in that area. In a study among schoolchildren in Bas-Congo, 32.1% were positive for schistosome infection (both *S. mansoni* and *S. haematobium*), and 56.4% for STHs infections (i.e: *Ascaris lumbricoides*, hookworm and *Trichiuris trichiura*), respectively [Linsuke et al: Schistosomiasis in schoolchildren of Kinshasa and Bas-Congo provinces, Democratic Republic of Congo, unpublished]. In 24 villages of the same area, 23.4% of the inhabitants above five years of age were positive for *T. solium* taeniasis (Madinga et al., 2017). Finally, in the village community of Malanga, a *T. solium* cysticercosis prevalence of 21% was reported (Kanobana et al., 2011). The current study is embedded in the latter study (Kanobana et al., 2011) and aims to investigate the epidemiology of co-infections with *T. solium*, *Schistosoma* spp and STHs at local level, herewith generating evidence to inform an integrated approach towards NTD control. We focus on prevalence and risk factors of helminth co-infections with *T. solium* taeniasis/cysticercosis as well as associations between different helminth species.

2. Methods

2.1. Ethical statements

This study was approved by the Ethical Committee of the University of Kinshasa, DRC, by the Institutional Review board of the Institute of Tropical Medicine in Antwerp, Belgium and by the Ethical Committee of the University of Antwerp, Belgium. Voluntary written informed consent was obtained from each participant before taking part in the study. For individuals below the consenting age (18 years), parents or legal guardians were asked to consent on their behalf on a separate consent form. Also literate children above 14 were requested to provide their own assent.

2.2. Study area and population

The field study was conducted in Malanga (5°33′S and 4°21′E), a village situated in the rural health district of Kimpese, Bas-Congo province in western DRC (Fig. 1). The village is divided in six administrative districts: Malanga gare (MG), Malanga Quartier 1 (MQ1), Malanga Quartier 2 (MQ2), Malanga Quartier 3 (MQ3), Camp Militaire (CM) and ICB (old wood factory settlement). Geographically, MG and ICB are separated from the rest of the village by a distance of approximately five kilometers. The village lies along two small rivers and water supply depends on the proximity to one of the following three water points: i) Water point A: situated along the first river, between MG and ICB; ii) Water point B: situated along the first river downstream of the water point A and close to MQ2 and MQ3 and iii) Water point C, situated along the second river and close to MQ1 and CM. Drinking water comes either from the river or from two artisanal water wells built in the vicinity of water points A and C. Subsistence farming and free-roaming pig husbandry are the main economic activities of the village inhabitants. Of 1250 individuals census population in 2009, a total of 943 (75.4%) inhabitants were willing to participate in the initial study (Kanobana et al., 2011). Up to the time period covered by the current study, no control activity was implemented in the study area. Currently, school-based mass treatment campaigns against schistosomiasis and STH infections are gradually being implemented throughout the country, following a national plan against NTDs adopted in 2012 (République Démocratique du Congo MdSP, 2012). These campaigns target school aged children (5–15 years) using praziquantel and albendazole while preschool children (under five) and pregnant women are systematically treated for STH infections only, using mebendazole, by child and mother health programs. So far, there is no national control strategy for *T. solium* taeniasis/cysticercosis.

2.3. Study design and data collection

The current study is based on a posteriori objectives and methods from an initial study which assessed prevalence and risk factors of *T. solium* cysticercosis in a village-community (Kanobana et al., 2011). The original study was a population-wide cross sectional survey, conducted in August 2009 and including all consenting villagers above

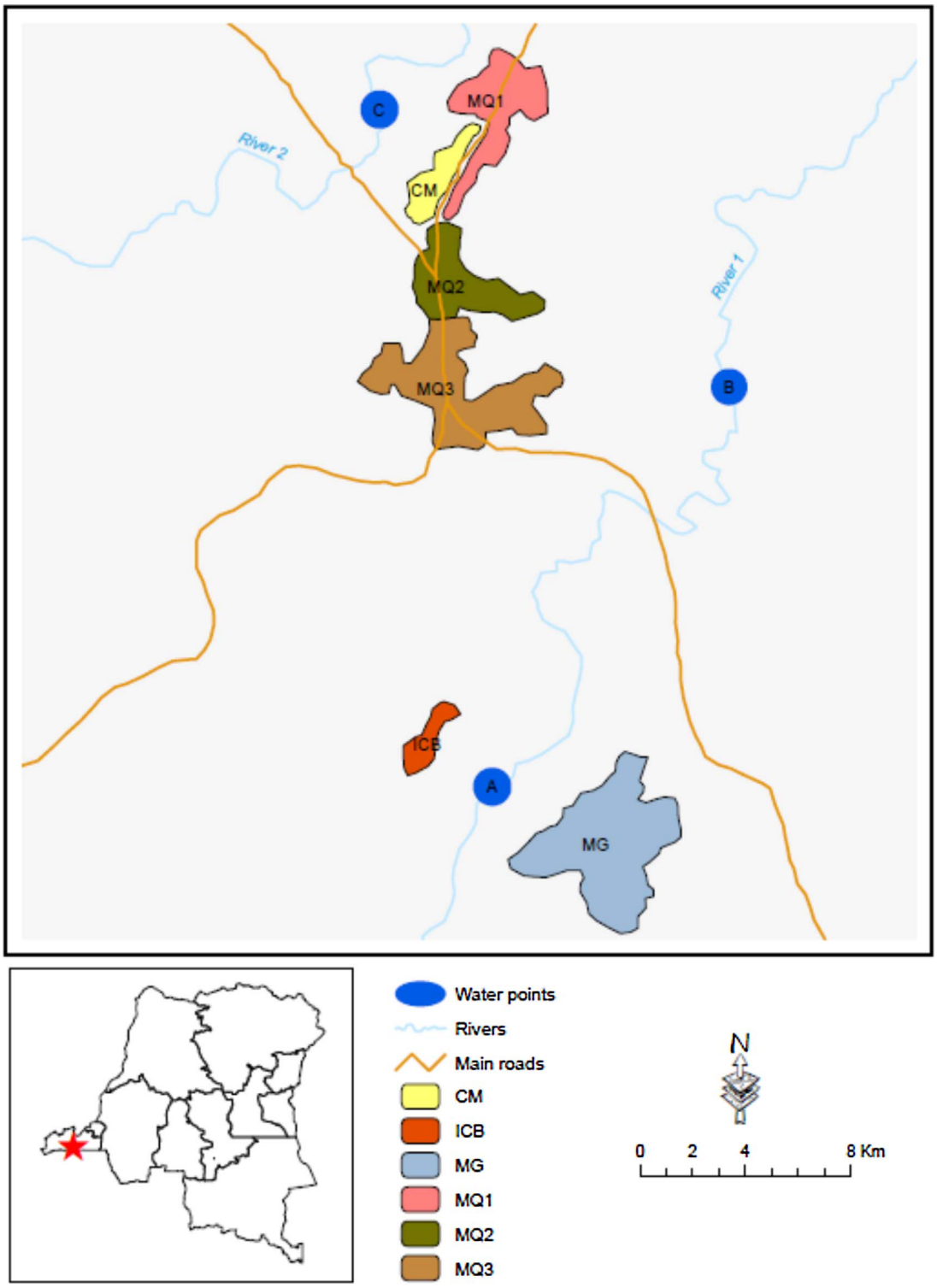

Fig. 1. Map of Malanga Village, displaying different village districts and water points.

1 year of age. In short: Upon informed consent, individual demographic (age, sex and district of residence) and hygiene-related (presence of toilet in the household, open defecation behavior, source of drinking water, rearing free roaming pigs) data were collected using a validated pretested questionnaire. In addition, stool, urine and 10 ml blood samples (maximum of 5 ml for children under 5 years) were collected from each participant. Biological samples were kept at 4 °C during the daily transfer from the field to the laboratory of Kimpese hospital. Upon arrival at the laboratory, urine samples were stored at −20 °C. Blood was allowed to clot overnight at 4 °C and then centrifuged at 3000 *g* for 15 min for serum collection. Serum was aliquoted into 1.8 ml vials and stored at −20 °C. Of each collected stool sample, an aliquot of approximately 1 g was mixed with 2 ml of 70% ethanol and stored at −20 °C. All samples were further transported under frozen condition to the Institute of Tropical Medicine, Antwerp and kept at −20 °C until use.

2.4. Sample size determination and subsampling strategy

Since the prevalence of *T. solium* co-infections in the study area was not known, the sample size for the current study was calculated on the basis of an expected prevalence of 50% as this produces a conservative estimate of the variance and hence the largest sample size (Macfarlane, 1997; Naing et al., 2006). Acceptable error was set at 5% with a level of confidence of 95% and a total population of 1250. Using a finite population correction, a minimal sample size of 295 was found to which 35 participants (12% of the sample size) were added in order to compensate for possible DNA extraction failure. Hence, the total subsample size was 330. Participants included in the original study were stratified by age (six groups), sex (two groups) and district of residence (six groups) for a total of 72 strata. The six age groups were the following: (1) preschool children (under five); (2) school-aged children (5–15 years); (3) adolescents and young adults (16–24 years); (4) adults-1 (25–39 years); (5) adults-2 (40–59 years) and (6) elderly people (60–80 years). This classification was justified by the need of including age category corresponding to age prevalence peak of the respective parasite species detected in the current study (Praet et al., 2010; Woolhouse, 1998). Subsequently, from these 72 stratified groups, a proportionate stratified random sub-sample of 330 individuals with complete data (biological samples and questionnaire data) was selected using the "random" command of Microsoft Excel® and included in the current study.

2.5. Laboratory analysis

Serum samples were tested for *T. solium* cysticercosis at the Institute of Tropical Medicine, Antwerp, using the B158/B60 monoclonal antibody-based antigen ELISA (Ag-ELISA) (Dorny et al., 2004). Ag-ELISA detects circulating antigens of the metacestode of *T. solium* and thus only individuals infected with living cysts. This assay has yielded 90% sensitivity and 98% specificity for the diagnosis of current cysticercosis in an endemic population and no cross-reactions were observed in sera from patients harboring infections with other helminths (i.e: *Ascaris*, *Schistosoma*, *Trichuris*, filaria, hydatid cysts) or protozoa (*Trypanosoma, Entamoeba, Plasmodium*) (Erhart et al., 2002). It was used as a measure of current *T. solium* cysticercosis. Seropositivity was defined as a ratio (i.e. optical density divided by cut off value) > 1. DNA extraction and real-time PCR analysis for STHs, *T. solium* and *Schistosoma* spp were performed in the Laboratory of Parasitology in Leiden University Medical Center, the Netherlands. Before DNA isolation, the ethanol was removed from stool samples by a washing procedure as described before (ten Hove et al., 2008). To optimize DNA yields an additional bead-beating step was performed. DNA was isolated using DNeasy 96 Blood (Verweij and Stensvold, 2014). Phocin herpes virus-1 (PhHV-1) was added to the lysis buffer in each sample as an internal control and virus-specific primers and detecting probe were included in each reaction mixture. Three different multiplex real-time PCR detection panels were used for parasite specific DNA detection of nine intestinal helminths: (i) *Ancylostoma* spp, *Necator americanus*, *Ascaris lumbricoides*, *Strongyloides stercoralis* (Wiria et al., 2010) (ii) *Schistosoma* spp, *Trichuris trichiura* (Wiria et al., 2010) and *(iii) Taenia solium*, *Taenia saginata* and *Hymenolepis nana* (Praet et al., 2013). Urine samples were tested using the same ITS2-based multiplex real-time PCR for the detection of *Schistosoma* DNA as the one used for the stool samples (Obeng et al., 2008). A custom-made Hamilton robot platform was used for the DNA isolation and setup of the PCR reactions. Amplification, detection and analysis were performed using the CFX real-time detection system (Bio-Rad Laboratories, USA). Samples were considered positive if a fluorescent signal above the background fluorescence was seen within the 50 amplification cycles. Negative and positive control samples for each parasite species were included in each PCR run.

2.6. Data analysis

Descriptive statistical analysis was applied to describe the characteristics of the study population as well as prevalence with 95% confidence intervals (CIs) of single and multiple helminth infections. *T. solium* taeniasis/cysticercosis was defined as positivity in either relevant stool PCR or serum ELISA. Co-infection was defined as any concomitant infection with at least two different helminth species. In this study, we focused on two combinations: *i*) STHs and *T. solium* taeniasis/cysticercosis and *ii*) *Schistosoma* and *T. solium* taeniasis/cysticercosis. This choice is justified by the need of bringing new insight for integrated control of *T. solium* taeniasis/cysticercosis with existing control strategies against schistosomiasis and STH infections, using common anthelminthics. The village districts that were close to each other were grouped in three categories as follows: i) MG and ICB, close to water point "A"; ii) MQ2 and MQ3, close to water point "B"; iii) MQ1 and CM, close to water point "C". For reasons of relevance to control programs, the age variable was aggregated in two categories: preschool and school aged children (1–15 years), representing the main target group of the control strategy against STH infections and schistosomiasis versus adolescents and adults (16–80 years). Bivariate (i.e. Pearson Chi-square and Fisher's exact test) and multivariate analysis (logistic regression) were used to assess risk factors of single and co-infections as well as pair wise associations between different helminth species. These analyses were done in R Studio version 0.98.1028 for Windows and the level of significance was set at alpha = 0.05.

3. Results

3.1. Description of the study population

Among the 330 subjects included, 148 (44.8%) were male and 182 (55.2%) were female. Age ranged from 1 to 80 years with a median of 18 years (IQR = 9–44 years). As in the original study (Kanobana et al., 2011), the majority of the participants were from MQ2 and MQ3 (56.7% of the sample population).

3.2. Prevalence of single and multiple helminth infections

PCR showed no inhibition of amplification in any of the samples as the cycle-threshold values of the internal PhHV-1 control were always within the expected range. All samples tested negative for *Ancylostoma* spp, *T. saginata* or *H. nana*. Among those included, 240 (72.7%) were infected with at least one helminth species; 109 (33%) harbored one helminth species and 131 (39.7%) harbored at least two species. The median number of parasite species per subject was one, ranging from zero to seven. Tables 1a and 1b show the percentages of PCR positives for STHs, *Schistosoma* spp and *T. solium* taeniasis as well as seropositives for *T. solium* cysticercosis, and for the two co-infections of interest, respectively. The percentage of positives for STHs infection was significantly higher [51.8% (95%CI: 46.3–57.2)] than that of *Schistosoma* spp infection [29.4% (95%CI: 24.6–34.7)] and *T. solium* taeniasis/cysticercosis [24.2% (95%CI: 19.8–29.3)]. For co-infections, the percentages of positives was 16.1% (95%CI: 12.4–20.6) for STHs-*Schistosoma*, 14.5% (95%CI: 11–18.9) for STHs-*T. solium* taeniasis/cysticercosis and 8.2% (95%CI: 5.6–11.8) for *Schistosoma-T. solium* taeniasis/cysticercosis, respectively. Fig. 2 shows the numbers of single and multiple helminth infections in a Venn diagram.

3.3. Risk factors of helminth co-infections

The risk factors for the respective helminth co-infections are summarized in Table 2. No significant associations were found between individual demographic or hygiene-related characteristics and *Schistosoma-T. solium* taeniasis/cysticercosis co-infections. Male sex (OR = 2

Table 1a
Overall prevalence (95%CI) of infection with STHs, *Schistosoma* spp and *T. solium* taeniasis/cysticercosis by individual demographic and hygiene-related characteristics.

Characteristics	Categories	n	STHs		*Schistosoma* spp		*T. solium* taeniasis/cysticercosis	
			Positive	% (95%CI)	Positive	% (95%CI)	Positive	% (95%CI)
All	All	330	171	51.8 (46.3–57.2)	97	29.4 (24.6–34.7)	80	24.2 (19.8–29.3)
Sex	Male	148	84	56.8 (48.4–64.9)	34	23 (16.5–30.6)	45	30.4 (23.1–38.5)
	Female	182	87	47.8 (40.4–55.3)	63	34.6 (27.7–42)	35	19.2 (13.8–25.7)
Age category	1-15 years	152	85	55.9 (47.6–64)	44	28.9 (21.9–36.8)	36	23.7 (17.2–31.3)
	16-80 years	178	86	48.3 (40.8–55.9)	53	29.8 (23.2–37.1)	44	24.7 (18.6–31.7)
Village districts	MG and ICB	61	36	59 (45.7–71.4)	27	44.3 (31.5–57.6)	12	19.7 (10.6–31.8)
	MQ2 and MQ3	187	99	52.9 (45.5–60.3)	45	24.1 (18.1–30.8)	37	19.8 (14.3–26.2)
	MQ1 and CM	82	36	43.9 (33–55.3)	25	30.5 (20.8–41.6)	31	37.8 (27.3–49.2)
Presence of a latrine in the household	No	139	73	52.5 (43.9–61)	36	25.9 (18.8–34)	39	28.1 (20.8–36.3)
	Yes	191	98	51.3 (44–58.6)	61	31.9 (25.4–39.1)	41	21.5 (15.9–28)
Open defecation behavior	Yes	287	147	51.2 (45.3–57.1)	82	28.6 (23.4–34.2)	76	26.5 (21.5–32)
	No	43	24	55.8 (39.9–7.09)	15	34.9 (21–50.9)	4	9.3 (2.6–22.1)
Source of drinking water	Well	70	40	57.1 (44.7–68.9)	30	42.9 (31.1–55.3)	14	20 (11.4–31.3)
	River	260	131	50.4 (44.1–56.6)	67	25.8 (20.6–31.5)	66	25.4 (20.2–31.1)
Rearing free roaming pigs	Yes	77	36	46.8 (35.3–58.5)	31	40.3 (29.2–52.1)	17	22.1 (13.4–33)
	No	253	135	53.4 (47–59.6)	66	26.1 (20.8–32)	63	24.9 (19.7–30.7)

(95%CI = 1.1–5), p = 0.03), residency of MQ1 and CM districts (OR = 3.2 (95%CI = 1.1–7.8), p = 0.03) as well as open defecation behavior (OR = 3.8 (95%CI = 1.1–6.5), p = 0.04) were associated with higher odds of co-infection with STHs and *T. solium* taeniasis/cysticercosis.

3.4. Pair wise associations between helminth species

Statistically significant pair wise associations between helminth species adjusted for age, sex and geographical location are given in Table 3. Significant pairwise associations were observed between STHs species and between *T. solium* cysticerci and two STHs, namely *N. americanus* (OR = 2.1 (95%CI = 1.3–3.7), p < 0.01) and *S. stercoralis* (OR = 2.9 (95%CI = 1.3–6.7), p = 0.02).

4. Discussion

This study investigated the epidemiology of co-infections with STHs, *Schistosoma* and *T. solium* taeniasis/cysticercosis in a co-endemic village community in DRC. Using PCR-based diagnosis we were able to detect at once a broader number of parasite species than has been done so far, bringing new insights in the epidemiology of helminth infections in the study area. For example, species diagnosis of hookworm and *Taenia* spp showed the presence of *N. americanus* and *T. solium* while *Ancylostoma* spp, *T. saginata* and *H. nana* were not found. Such species identification could not be performed in previous studies as they assessed STH and *Taenia* spp infections using microscopy. Moreover, our study reported for the first time the presence of *S. stercoralis* in the study area. Prior to our study, only two studies have reported *S. stercoralis* infection elsewhere in DRC (Henry et al., 1995; Prinz et al., 1979). Despite its better performance compared to microscopy, the PCR assay used in the current study did not allow for species diagnosis of *Schistosoma* spp. However, the nature of the sample can be considered indicative of the species (*S. mansoni* in stool and *S. haematobium* in urine), although ectopic infections cannot be excluded. It should also be noted that PCR is not yet a routine diagnostic method especially in low

Table 1b
Prevalence (95%CI) of co-infections with STHs, *Schistosoma* spp and *T. solium* taeniasis/cysticercosis by individual demographic and hygiene-related characteristics.

Characteristics	Categories	n	STHs-*Schistosoma*		STHs-*T. solium* taeniasis/cysticercosis		*Schistosoma*-*T. solium* taeniasis/cysticercosis	
			Positive	% (95%CI)	Positive	% (95%CI)	Positive	% (95%CI)
All	All	330	53	16.1 (12.4–20.6)	48	14.5 (11–18.9)	27	8.2 (5.6–11.8)
Sex	Male	148	23	15.5 (10.1–22.4)	29	19.6 (13.5–26.9)	12	8.1 (4.3–13.7)
	Female	182	30	16.5 (11.4–22.7)	19	10.4 (6.4–15.8)	15	8.2 (4.7–13.2)
Age category	1-15 years	152	28	18.4 (12.6–25.5)	19	12.5 (7.7–18.8)	14	9.2 (5.1–15)
	16-80 years	178	25	14 (9.3–20)	29	16.3 (11.2–22.6)	13	7.3 (3.9–12.2)
Village districts	MG and ICB	61	15	24.6 (14.5–37.3)	5	8.2 (2.7–18.1)	4	6.6 (1.8–15.9)
	MQ2 and MQ3	187	25	13.4 (8.8–19.1)	23	12.3 (8–17.9)	13	7 (3.8–11.6)
	MQ1 and CM	82	13	15.9 (8.7–25.6)	20	24.4 (15.6–35.1)	10	12.2 (6–21.3)
Presence of a latrine in the household	No	139	22	15.8 (10.2–23)	25	18 (12–25.4)	9	6.5 (3–11.9)
	Yes	191	31	16.2 (11.3–22.2)	23	12 (7.8–17.5)	18	9.4 (5.7–14.5)
Open defecation behavior	Yes	287	44	15.3 (11.4–20)	46	16 (12–20.8)	26	9.1 (6–13)
	No	43	9	20.9 (10–36)	2	4.7 (0.6–15.8)	1	2.3 (0.1–12.3)
Source of drinking water	Well	70	18	25.7 (16–37.6)	6	8.6 (3.2–17.7)	4	5.7 (1.6–14)
	River	260	35	13.5 (9.6–18.2)	42	16.2 (11.9–21.2)	23	8.8 (5.7–13)
Rearing free roaming pigs	Yes	77	14	18.2 (10.3–28.6)	8	10.4 (4.6–19.4)	6	7.8 (2.9–16.2)
	No	253	39	15.4 (11.2–20.5)	40	15.8 (11.5–20.9)	21	8.3 (5.2–12.4)

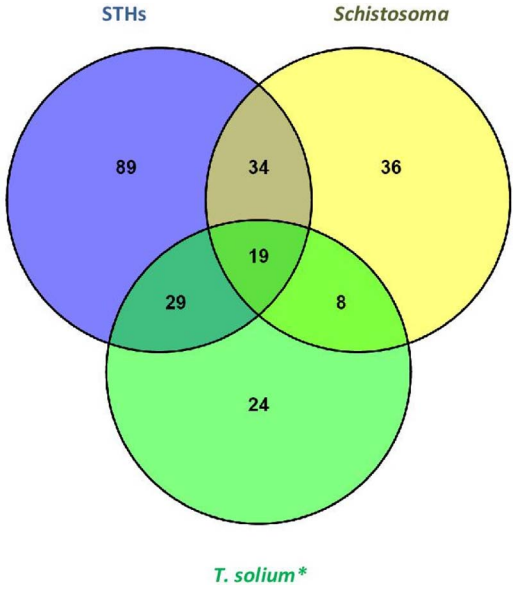

Fig. 2. Venn diagram showing numbers (n) of single and multiple infections with STHs, *Schistosoma* spp and *T. solium*. (*): both *T. solium* taeniasis and cysticercosis.

income settings since reagents and equipment are expensive and trained people often lacking.

Village districts of MQ1 and MC were associated with higher risk of co-infection with STHs and *T. solium* taeniasis/cysticercosis. These same village districts were also associated with higher risk of single infection with *T. solium* taeniasis/cystocercosis (see complementary data). This is in line with previous studies, which found that the distribution of co-infections was determined by (factors underlying) the distribution of the rarest species or the species with the most restrictive life-cycle. So far, this has only been studied for STHs-*Schistosoma* spp (Brooker and Clements, 2009; Soares Magalhaes et al., 2011; Yajima et al., 2011) and helminths-*plasmodium* co-infections (Brooker et al., 2006; Brooker et al., 2012). Our findings suggest that this is also for co-infections with STHs and *T. solium* taeniasis/cysticercosis in our study area, since STH infections were more prevalent and more randomly distributed than *T. solium* taeniasis/cysticercosis.

Not surprisingly, STHs-*T. solium* taeniasis/cysticercosis co-infections were associated with open defecation behavior. Indeed, lack of adequate sanitation is a major risk factor for STH infections and also for *T. solium* cysticercosis (Coral-Almeida et al., 2015; Strunz et al., 2014), and hence also for their co-infections. Interventions such as improved latrines and 'Water, Sanitation and Hygiene' (WASH) need to be promoted, both for single as well as for co-infected people.

Moreover, male participants were more likely to be co-infected with STHs and *T. solium* taeniasis/cysticercosis than female participants, following the distribution of *T. solium* taeniasis/cysticercosis. Hormonal, behavioral/occupational and/or biological differences have been reported as underlying factors of sex-related differences in single infections (Klein, 2004). Further studies are needed to better understand the sex-related patterns of co-infections, so as to further fine-tune target groups for integrated treatment in co-endemic areas.

An interesting finding of this study is the significant pair wise associations between *T. solium* cysticercosis and two STHs species (*N. americanus* and *S. stercoralis*). To our knowledge, these associations have not yet been reported in literature to date. STHs infections are associated with a strong Th2 and significant suppression of Th1 type immune response (Loukas and Prociv, 2001).This Th2 response may increase the susceptibility to *T. solium* cysticercosis infection. Yet, not enough is known about the underlying immunological mechanisms of exposure to *T. solium* and the development of (clinical) neurocysticercosis (Chavarria et al., 2003; Lopez-Moreno, 2002; Terrazas et al., 1999), let alone for co-infections with *T. solium* cysticercosis, and further studies are needed. This finding might have an implication in terms of integrated control. It has been reported that a triple-dosis of ablendazole (400 mg/day for 3 consecutive days) or mebendazole (500 mg/day for 3 consecutive days) can significantly reduce the prevalence of both taeniasis and STH infections at the same time (Ash et al., 2015; Steinmann et al., 2011). Such an approach can be recommended in our study area, since anthelmintic treatment of human tapeworm carriers destroys the source of infection and prevents both human and porcine cysticercosis.

No significant association was found between *Schistosoma-T. solium* taeniasis/cysticercosis co-infection and risk factors studied. Also the respective single infections had no common risk factors (see supplementary data). Nevertheless, in areas where schistosomiasis and *T. solium* taeniasis/cysticercosis coexist, WHO recommends to integrate their control as both diseases can be treated with praziquantel at a dose of 40 mg/kg for schistosomiasis and 5–10 mg/kg for taeniasis (World Health Organisation, 2012). However, the safety of mass administration of praziquantel in communities where *T. solium* cysticercosis, and thus possibly neurocysticercosis, exist is yet to be assessed. Anti-parasitic therapy of neurocysticercosis with praziquantel can trigger inflammatory reactions, exacerbating neurologic symptoms, with a risk of intracranial hypertension, which can be fatal (Flisser et al., 1993). Close follow up is therefore required when using praziquantel in such areas.

We are aware that our study has some limitations. Indeed, these are a posteriori data from a study which was initially designed to study epidemiology of human cysticercosis in one selected village. Although we checked for several risk factors, we cannot exclude that other unknown or unmeasured biological, behavioral or environmental risk factors may have been of influence as well, and even act as confounding factors, thereby influencing the study results. Intensity of helminth infections, which is a relevant indicator for helminth-related morbidity and control strategy, was not investigated in the current study.

Table 2
Risk factors of co-infections with *T. solium* taeniasis/cysticercosis, STHs and *Schistosoma* spp.

Variables	Categories	STHs-*Schistosoma* spp		STHs-*T. solium* taeniasis/cysticercosis		*Schistosoma* spp − *T. solium* taeniasis/cysticercosis	
		OR (95%CI)	p	AOR (95%CI)	p	AOR (95%CI)	p
Sex	Male	–	–	1	–	–	–
	Female	–	–	0.5 (0.2–0.9)	**0.03**	–	–
Village districts	MG and ICB	1		1		–	–
	MQ2 and MQ3	0.4 (0.2–0.9)	**0.04**	1.4 (0.5–3.9)	0.5	–	–
	MQ1 and CM	0.5 (0.2–1.3)	0.29	3.2 (1.1–7.8)	**0.03**	–	–
Open defecation behavior	No	–	–	1		–	–
	Yes	–	–	3.8 (1.1–6.5)	**0.04**	–	–

AOR: Adjusted Odds ratio.

Table 3
Significant pair-wise associations between helminth species.

Parasite	Bivariate			Multivariate		
	Association	OR (95%CI)	p	Association	AOR (95%CI)	p
A. lumbricoides	*N. americanus*	4 (2.3–7)	< 0.001	*N. americanus*	3.2 (1.7–6)	< 0.001
	T. trichiura	3.6 (2–6.5)	< 0.001	*T. trichiura*	3 (1.4–5.8)	< 0.01
	S. stercoralis	8 (3.4–19)	< 0.001	*S. stercoralis*	6 (4.2–11.4)	< 0.001
N. americanus	*A. lumbricoides*	4 (2.3–7)	< 0.001	*A. lumbricoides*	4 (2.3–7.1)	< 0.001
	T. trichiura	3 (1.7–5.5)	< 0.001	*T. trichiura*	3.2 (1.7–5.8)	0.004
	S. stercoralis	3 (1.3–6.8)	0.01	*S. stercoralis*	2.8 (1.2–6.5)	0.01
	T. solium Cysticerci	2.1 (1.3–3.7)	0.01	*T. solium cysticerci*	3.2 (1.7–5.8)	< 0.01
T. trichiura	*A. lumbricoides*	3.6 (2–6.5)	< 0.001	*A. lumbricoides*	4 (2.1–7.5)	< 0.001
	N. americanus	3.1 (1.7–5.5)	< 0.001	*N. americanus*	3.2 (1.7–5.8)	0.005
	S. stercoralis	2.7 (1.1–6.3)	0.03	*S. stercoralis*	2.8 (1.2–7)	0.02
S. stercoralis	*A. lumbricoides*	8 (3.4–19)	< 0.001	NA		
	N. americanus	3 (1.3–6.8)	0.01			
	T. trichiura	2.7 (1.1–6.3)	0.03			
	T. solium cysticercosis	2.9 (1.3–6.7)	0.01			
T. solium cysticerci	*N. americanus*	2.1 (1.3–3.7)	0.01	*N. americanus*	2.2 (1.2–3.8)	< 0.01
	S. stercoralis	2.9 (1.3–6.7)	0.01	*S. stercoralis*	2.7 (1.1–6.5)	0.02

AOR: Odds ratio adjusted for age, sex and village district. NA: not applicable (i.e.: less than 10 positive per independent variable).

5. Conclusion

The current study shows that co-infections with STHs, *Schistosoma* spp and *T. solium* are common in this polyparasitic community. Risk factors for co-infection with STHs and *T. solium* taeniasis/cysticercosis were similar to those for single *T. solium* taeniasis/cysticercosis. Significant associations were found between *T. solium* cysticercosis and infections with *N. americanus* as well as *S. stercoralis*. Our findings may contribute to design an integrated control of *T. solium* taeniasis/cystcercosis with existing strategies against schistosomiasis and STH infections. More knowledge about the distribution and dynamics of helminth (co-)infections will contribute to the improvement of the prevention and control of polyparasitism in DRC and elsewhere.

Competing interest

The authors declare that they have no competing interest.

Acknowledgements

The authors would like to thank the team of the health district of Kimpese for its contribution to the field; Bjorn Victor and Famke Janssen for their assistance with the Ag-ELISA, Daniel Bimmel and Rebecca van Grootveld for performing the PCR analysis. This work was funded by the Belgian Development Cooperation within the Framework Agreement Programme 3 between the Institute of Tropical Medicine, Antwerp and the Belgian Development Cooperation. A travel grant for Kirezi Kanobana was obtained from the Fonds Wetenschappelijk Onderzoek Vlaanderen. Joule Madinga is a PhD student supported by the « developing cooperation » scholarship of the Université catholique de Louvain. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.03.019.

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