Schistosoma mansoni and Schistosoma haematobium infection and morbidity in a co-endemic focus: Integrated study of epidemiological, micro-geographical and immunological patterns

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Chapter 2

Epidemiology of mixed *Schistosoma mansoni* and *Schistosoma haematobium* infections in northern Senegal

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Abstract

Due to the large overlap of *Schistosoma mansoni*- and *Schistosoma haematobium*-endemic regions in Africa, many people are at risk of co-infection, with potential adverse effects on schistosomiasis morbidity and control. Nonetheless, studies on the distribution and determinants of mixed *Schistosoma* infections have to date been rare. We conducted a cross-sectional survey in two communities in northern Senegal (n=857) to obtain further insight into the epidemiology of mixed infections and ectopic egg elimination. Overall prevalences of *S. mansoni* and *S. haematobium* infection were 61% and 50% respectively, in these communities. Among infected subjects, 53% had mixed infections and 8% demonstrated ectopic egg elimination. Risk factors for mixed infection - i.e. gender, community of residence and age - were not different from what is generally seen in *Schistosoma*-endemic areas. Similar to overall *S. mansoni* and *S. haematobium* infections, age-related patterns of mixed infections showed the characteristic convex-shaped curve for schistosomiasis, with a rapid increase in children, a peak in adolescents, and a decline in adults. Looking at the data in more detail however, the decline in overall *S. haematobium* infection prevalences and intensities appeared to be steeper than for *S. mansoni*, resulting in a decrease in mixed infections and a relative increase in single *S. mansoni* infection with age. Moreover, individuals with mixed infections had higher infection intensities of both *S. mansoni* and *S. haematobium* than those with single infections, especially those with ectopic egg elimination ($p<0.05$). High infection intensities in mixed infections, as well as age-related differences in infection patterns between *S. mansoni* and *S. haematobium*, may influence disease epidemiology and control considerably, and merit further studies into the underlying mechanisms of *Schistosoma* infections in co-endemic areas.

Introduction

Schistosomiasis is amongst the most common human parasitic diseases with an estimated 207 million people infected worldwide. More than 90% of them live in sub-Saharan Africa (Hotez and Kamath, 2009). The global distribution map of *Schistosoma* shows a large overlap of *Schistosoma mansoni*- and *Schistosoma haematobium*-endemic areas in Africa (Doumenge et al., 1987; Gryseels et al., 2006; Montgomery, 2011), indicating that many people are at risk of co-infection with both species. Nevertheless, little is known about the distribution and determinants of such mixed infections in human populations (Dennis et al., 1983; Robert et al., 1989; Ahmed et al., 1996; Booth et al., 1998; Lwambo et al., 1999). Animal models have described that *S. mansoni* and *S. haematobium* interact in the host. The two species have been shown to form heterologous male-female pairs with the male determining the oviposition site and the female producing eggs characteristic of her species (Khalil and Mansour, 1995; Southgate et al., 1998; Webster et al., 1999). This phenomenon probably contributes to the occurrence of ectopic egg elimination, i.e. *S. mansoni* eggs in urine or *S. haematobium* eggs in feces, in mixed foci (Ratard et al., 1991; Cunin et al., 2003).

Recently, differences have been observed between single and mixed infections regarding their association with bladder as well as liver pathology (Koukounari et al., 2010). Also, unforeseen increases in *S. mansoni* infection have been observed after praziquantel treatment in co-endemic areas (Ernould et al., 1999a; Koukounari et al., 2010). Moreover, mixed infections may lead to the hybridization of *Schistosoma* spp. or parthenogenesis (Jourdane et al., 1995; Khalil and Mansour, 1995), with as yet unclear consequences for disease and transmission (Wright and Ross, 1980; Webster and Southgate, 2003; Huyse et al., 2009). Understanding the epidemiology of mixed infections will help us to answer important standing questions on the underlying mechanisms towards morbidity and to develop effective strategies for the prevention and control of schistosomiasis in co-endemic areas.

During the past decades, many communities in northern Senegal have become co-endemic for *S. mansoni* and *S. haematobium* (De Clercq et al., 1999; Ernould et al., 1999a; Southgate et al., 2001; Van der Werf et al., 2002; Ten Hove et al., 2008; Huyse et al., 2009). *Schistosoma mansoni* was introduced in Richard-Toll in 1988 upon construction of the Diama dam and rapidly spread throughout the region (Talla et al., 1990; , 1992). By 1994, virtually the whole Lac de Guiers area had become exposed to this species (Picquet et al., 1996). Today, both *S. mansoni* and *S. haematobium* are widespread, resulting in a large number of people with mixed infections in the communities around the lake.

Here, we report the results of a cross-sectional study investigating the epidemiology of mixed *Schistosoma* infections in two communities on the banks of Lac de Guiers in

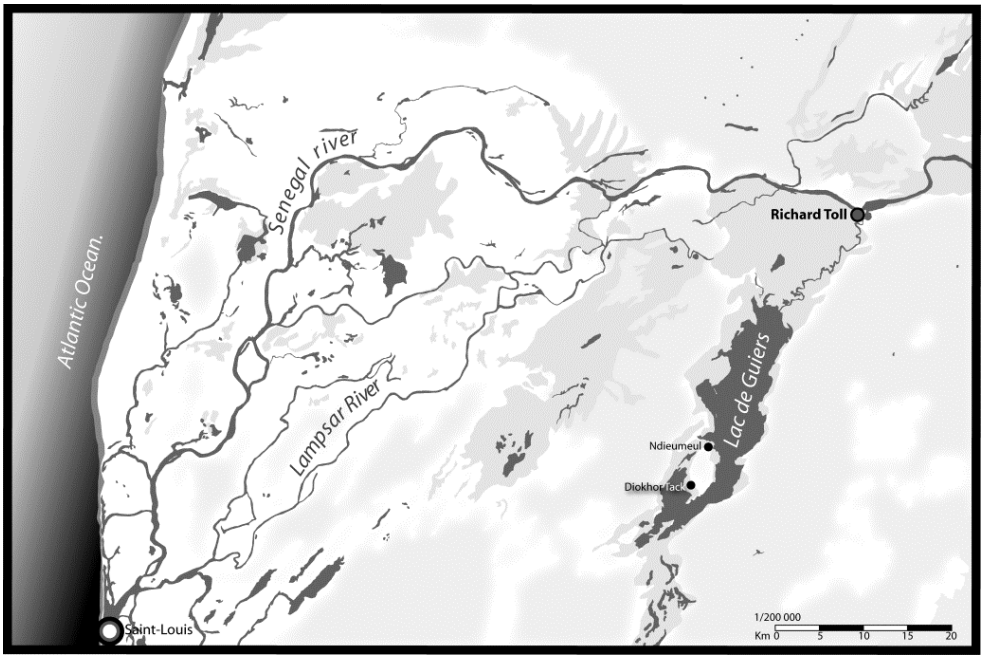

Figure 2.1. Map of northern Senegal indicating the communities participating in this study, Ndieumeul and Diokhor Tack.

northern Senegal. We studied the patterns of *S. mansoni* and *S. haematobium* infection in these mixed foci, and compared mixed with single infections. Possible underlying mechanisms and the impact of the reported findings are discussed.

Material and methods

Study area

Ndieumeul (also known as Thiekène; 16°13'12"N 15°51'36"W) and Diokhor Tack (16°11'24"N 15°52'48"W), are the largest communities on the Nouk Pomo peninsula in Lac de Guiers, Senegal and are situated 4 km apart (Figure 2.1). These Wolof communities have a total estimated population size of 1,300 people. Cultivation is the main means of subsistence and the farmlands are irrigated with water from the lake. Although the water from Lac de Guiers is piped to the capital city of Dakar, 250 km away (Berger et al., 2006), the people living nearby do not have access to safe water. To our knowledge, there have been no periodic anthelminthic treatment programs in these villages prior to our study.

The present study was conducted in 2009 as part of a larger investigation on the immuno-epidemiology of *Schistosoma* infection and morbidity (SCHISTOINIR:

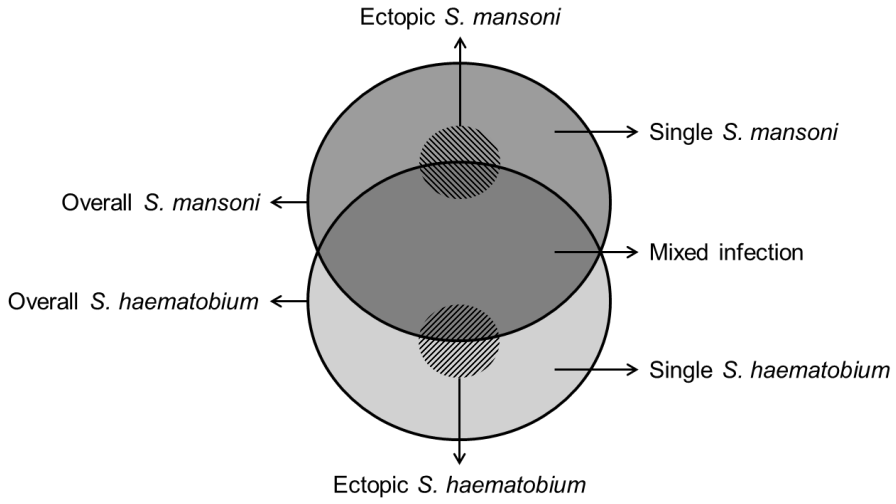

Figure 2.2. Schematic overview of *Schistosoma* infection groups.

www.york.ac.uk/res/schistoinir) for which approval was obtained by the review board of the Institute of Tropical Medicine in Belgium, the ethical committee of the Antwerp University Hospital in Belgium and 'Le Comité National d'Ethique de la Recherche en Santé' in Senegal.

Informed and written consent were obtained from all participants. After the study, praziquantel (40 mg/kg) and mebendazole (500 mg) treatment were offered to all community members to treat and prevent schistosomiasis and soil-transmitted helminthiasis, respectively, according to WHO guidelines (WHO, 2006).

Parasitology

Two feces and two urine samples were collected from each participant on consecutive days. For each feces sample, two Kato-Katz slides of 25 mg of fecal material each were prepared and microscopically examined for *Schistosoma* spp., *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (Katz et al., 1972; WHO, 1991). *Schistosoma mansoni* infection intensity was expressed as the number of eggs detected per gram of feces (epg). Urine filtration was performed using a filter of 12µm pore-size (Isopore, USA) according to standard procedures (WHO, 1991). *Schistosoma haematobium* infection intensity was expressed as the number of eggs detected per 10 ml of urine (ep10ml). World Health Organization (WHO) standards were used to categorize schistosomal infection intensity into light (1-99 epg for *S. mansoni* and 1-49 ep10ml for *S. haematobium*), moderate (100-399 epg for *S. mansoni*) and heavy infections (≥400 epg for *S. mansoni* and ≥50 ep10ml for *S. haematobium*) (Montresor et al., 1998). Ectopic eggs were measured qualitatively (positive/negative).

In this paper, the following definitions are used (see also Figure 2.2): single infection is defined as passing eggs of only one species, and mixed infection as passing eggs of both *S. mansoni* and *S. haematobium.* Ectopic egg elimination refers to the elimination of schistosomal eggs via the unusual route – i.e. *S. haematobium* eggs in feces or *S. mansoni* eggs in urine. Overall *S. mansoni* infection refers to both mixed and single *S. mansoni* infections. Overall *S. haematobium* infection includes both mixed and single *S. haematobium* infections.

Statistical analyses

IBM SPSS 19.0 (SPSS, Inc.) was used for statistical analyses. Results were considered significant when the *p*-value was <0.05. Data were characterized by percentages, geometric means, and 95% confidence intervals (CIs). As egg output showed skewed distributions, data were normalized by 10log-transformation. Geometric means of egg counts (GM epg or ep10ml) were calculated for microscopically positive individuals to analyze the intensity of infection. The Mann-Whitney U test was used to determine age differences between the communities. The Pearson Chi-square test was used to determine the association between community and gender as well as between *S. mansoni* and *S. haematobium* infection status. The independent-samples T-test was used to compare GM infection intensities between single and mixed infections.

Multivariable logistic regression models were used to identify independent risk factors for overall *S. mansoni* and overall *S. haematobium* infection, respectively. In the first model, *S. mansoni*-positive subjects were compared with *S. mansoni*-negative subjects. In the second model, *S. haematobium*-positive subjects were compared with *S. haematobium*-negative subjects. Age, gender and community of residence were included as potential risk factors. Similarly, the association between mixed infection and these risk factors was assessed, with single *S. mansoni* and single *S. haematobium* infection as reference groups, respectively.

To assess the association between infection intensity (*S. mansoni* or *S. haematobium* infection) and mixed infection, multivariable linear regression was performed, using a dummy variable for mixed infection (1=mixed, 0=single), and age, gender and community of residence as other risk factors.

The association between ectopic egg elimination and intensity of infection in mixed infections was assessed by multivariable logistic regression, with non-ectopic mixed infections as a reference group. Infection intensity of *S. mansoni* and *S. haematobium*, age, gender, and community of residence were included as risk factors.

Due to the skewed trend of infection prevalences and intensities with age, the population was divided into seven age groups (0-4, 5-9, 10-14, 15-19, 20-29, 30-39 and ≥ 40 years) in all models. In addition, significant interaction terms ($p<0.05$) were added to the equations.

Results

Complete data (based on ≥1 feces sample and ≥10 ml of urine) were obtained from 857 individuals. This group consisted of 428 males and 429 females, 253 subjects from Ndieumeul and 604 from Diokhor Tack, with a median age of 16 (range 0-85) years. There were no significant differences regarding age or gender between the two communities (p>0.8).

Seventy-three per cent of the study population was infected with at least one *Schistosoma* spp. (Table 2.1). The overall prevalence of *S. mansoni* infection was 61% (520/857) and that of *S. haematobium* 50% (431/857), with 15% (129/857) and 9% (76/857) of heavy infections, respectively. Among infected subjects, 53% (328/623) had mixed infections: 8% (49/623) had mixed infections with ectopic egg elimination and 45% (279/623) without ectopic egg elimination.

Table 2.1. Schistosomal infection prevalences and intensities.

Schistosoma mansoni infection		*Schistosoma haematobium* infection		Prevalence n (%)		*S. mansoni* infection intensity		*S. haematobium* infection intensity	
feces	urine	feces	urine			GM epg (95%CI)		GM ep10ml (95%CI)	
Positive subjects				**623**	**(72.7)**				
Single infections				**295**	**(34.4)**				
+	-	-	-	191	(22.3)	76.6	(62.0-94.4)		
-	-	-	+	102	(11.9)			2.3	(1.8-3.0)
-	-	+	-	1	(0.1)				
-	+	-	-	1	(0.1)				
Mixed infections				**328**	**(38.3)**	**167.4**	**(142.0-197.2)**	**9.9**	**(8.0-12.1)**
without ectopic eggs				279	(32.6)				
+	-	-	+	279	(32.6)	148.9	(124.7-177.7)	7.6	(6.1-9.5)
with ectopic *S. haematobium* eggs				2	(0.2)				
+	-	+	+	1	(0.1)	420.0		25.0	
+	-	+	-	1	(0.1)	60.0			
with ectopic *S. mansoni* eggs				47	(5.5)				
+	+	-	+	39	(4.6)	383.1	(264.2-554.5)	58.3	(36.2-93.9)
-	+	-	+	8	(0.9)			9.5	(1.7-48.7)
Negative subjects				**234**	**(27.3)**				
-	-	-	-	234	(27.3)				
Overall *S. mansoni* infections				520	(60.7)	125.3	(109.7-143.1)		
Overall *S. haematobium* infections				431	(50.3)			7.1	(5.9-8.4)
Total				**857**	**(100%)**				

GM: geometric mean; 95% CI: 95% confidence interval; epg: number of *Schistosoma mansoni* eggs / gram feces; ep10ml: number of *S. haematobium* eggs / 10 ml of urine.

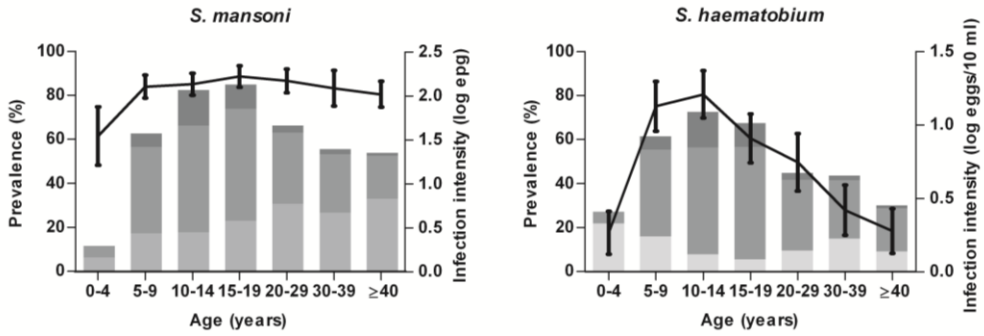

Figure 2.3. Age-prevalence and -intensity curves for schistosomal infection in the communities studied.

The bars indicate overall infection prevalences per age group. Dark grey stacks indicate infections with ectopic egg elimination. Grey stacks in the middle indicate mixed infections, and lighter grey stacks designate single *Schistosoma mansoni* and single *Schistosoma haematobium* infections (all without ectopic egg elimination). Lines indicate mean 10log-transformed infection intensities among positive subjects with 95% confidence intervals (whiskers). Epg: number of *S. mansoni* eggs detected per gram of feces; ep10 ml: number of *S. haematobium* eggs detected per 10 ml of urine.

Individuals who were positive for *S mansoni* were more likely to be infected with *S. haematobium* and vice versa ($p<0.001$). Among positive subjects, *S. mansoni* and *S. haematobium* infection intensities were significantly higher in mixed compared with single infections ($p<0.001$).

Furthermore, 2.5% (21/857) of the study population harbored one or more intestinal helminths. *Ascaris lumbricoides* was found in 17 and *T. trichiura* in six individuals. No hookworm infections were detected.

Mixed *Schistosoma* infections

Figure 2.3 indicates that overall *S. mansoni* infection prevalences were higher than those for *S. haematobium* in all age groups, except in children under five years. Accordingly, single *S. haematobium* infection was the most dominant infection in the youngest subjects (< 5 years). The prevalences and intensities of overall *S. mansoni* and *S. haematobium* infection, as well as mixed infections, increased up to the second decade of life, with a subsequent decrease in adults (Figure 2.3). The decline in prevalence and intensity was sharper and occurred at an earlier age for *S. haematobium* than for *S. mansoni*. As a result, single *S. mansoni* infection was the most dominant infection in the oldest age group (≥40 years). These patterns were similar in the two communities. Table 2.2 summarizes the risk factors for overall *S. mansoni* and *S. haematobium* infection, respectively. Age and community of residence were strongly

Table 2.2. Results from multivariable logistic models examining risk factors of overall *Schistosoma mansoni*, overall *Schistosoma haematobium*, as well as mixed infections.

Risk factors		Overall infection				Mixed infection			
		S. mansoni-positive versus *S. mansoni*-negative		*S. haematobium*-positive versus *S. haematobium*-negative		Mixed infection versus single *S. haematobium*		Mixed infection versus single *S. mansoni*	
		n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
Age [a]	0-4 years	96	0.02 (0.01-0.1)***	96	0.1 (0.03-0.2)***	26	0.02 (0.004-0.1)***	11	0.1 (0.01-1.0)
	5-9 years	163	0.3 (0.2-0.6)**	163	0.6 (0.3-1.4)	100	0.2 (0.1-0.7)**	102	0.8 (0.3-1.9)
	10-14 years	142	0.9 (0.4-1.8)	142	0.8 (0.3-1.7)	103	0.7 (0.2-2.0)	116	0.7 (0.3-1.8)
	15-19 years	92	Ref.	92	Ref.	62	Ref.	78	Ref.
	20-29 years	127	0.3 (0.2-0.6)**	127	0.1 (0.1-0.3)***	57	0.2 (0.1-0.8)*	84	0.2 (0.1-0.5)***
	30-39 years	94	0.2 (0.1-0.5)***	94	0.1 (0.05-0.3)***	41	0.1 (0.04-0.4)**	52	0.1 (0.02-0.3)***
	≥40 years	143	0.2 (0.1-0.4)***	143	0.1 (0.05-0.3)***	42	0.2 (0.1-0.6)**	77	0.1 (0.04-0.4)***
Gender	Male	428	Ref.	428	Ref.	202	Ref.	256	Ref.
	Female	429	1.2 (0.9-1.7)	429	0.5 (0.2-1.2)	229	1.5 (0.9-2.4)	264	0.5 (0.2-1.4)
Community	Ndieumeul	253	Ref.	253	Ref.	168	Ref.	190	Ref.
	Diokhor	604	0.4 (0.2-0.5)***	604	0.3 (0.2-0.5)***	263	0.4 (0.3-0.7)**	330	0.5 (0.3-0.7)***
Interaction	Age*Gender		N/A	857	***		N/A	520	*

Ref.: reference category; OR: odds ratio; 95% CI: 95% confidence interval; N/A, not applicable. *: $p<0.05$; **: $p < 0.01$; ***: $p < 0.001$.

[a] Associations with age were significant at the level of $p<0.001$.

associated with both infections. For *S. haematobium* infection, gender differences were more pronounced in adults than in children (p=0.008 for the age*gender interaction term); age-stratified analysis indicated that adult women (≥20 years of age) were more at risk of being infected with *S. haematobium* compared with their male counterparts (odds ratio (OR) 2.5; 95% CI: 1.6-4.0). A similar trend was found for *S. mansoni* infection, although it was not significant. The risk factors for mixed infection are summarized in Table 2.2, and reflect those of overall *S. mansoni* and *S. haematobium* infection. Again, gender differences were more pronounced in adults than in children. Age-stratified analysis showed that adult women were more at risk of being infected with mixed infections than their male counterparts (OR=2.7; 95% CI: 1.5-4.9 for mixed infection versus single *S. mansoni*, and OR=1.6; 95% CI: 0.7-3.7 for mixed infection versus single *S. haematobium*).

Similar risk factors were identified for infection intensities as for infection prevalences (data not shown). In addition, *S. mansoni* infection intensity was associated with the presence of *S. haematobium* infection and vice versa (standardized β=0.21 and 0.22 for *S. mansoni* and *S. haematobium* infection intensity, respectively (p<0.001)). These associations were similar in both communities.

Ectopic *Schistosoma* egg elimination

Ectopic *S. mansoni* egg elimination was found in 48 subjects and ectopic *S. haematobium* egg elimination in three subjects. Within the first group, most individuals also had *S. mansoni* eggs in feces and *S. haematobium* eggs in urine (n = 39, Table 2.1). This combination was restricted to 5-29-year-old individuals. Within this age range, ectopic elimination of *S. mansoni* eggs was significantly associated with schistosomal infection intensities: the OR for ectopic *S. mansoni* elimination was 3.5 (95%CI: 2.0-6.2) for a 10-fold increase in *S. haematobium* and 2.2 (95%CI: 1.1-4.4) for a 10-fold increase in *S. mansoni* infection intensity. Similarly, the average *S. haematobium* infection intensity was four times lower in those with single *S. haematobium* infection (GM=2.3 eggs/10 ml) than in those with additional ectopic *S. mansoni* eggs (GM=9.5 eggs/10 ml), suggesting a positive association between ectopic *S. mansoni* egg elimination and *S. haematobium* infection intensity in the other groups as well (Table 2.1).

Discussion

Due to the large overlap of *S. mansoni*- and *S. haematobium*-endemic regions in Africa, many people are at risk of co-infection, with potential adverse effects on schistosomiasis morbidity and control (Cheever et al., 1977; Friis et al., 1996; Koukounari et al., 2010). Nonetheless, studies on the distribution and determinants of mixed infections have to date been rare. Here, we report on the epidemiology of mixed *S.*

mansoni and *S. haematobium* infections as well as ectopic egg elimination in two mixed foci in northern Senegal.

The age-related patterns of overall *S. mansoni* and *S. haematobium* infection corresponded to the characteristic convex-shaped curve for schistosomiasis, with a peak in adolescence (Woolhouse, 1998; Cook and Zumla, 2009). Mixed infections followed the same pattern. However, a more detailed analysis revealed that the decline in overall *S. haematobium* infection prevalences and intensities after adolescence appeared to be steeper than for *S. mansoni*. Previous studies in either *S. mansoni*- or *S. haematobium*-endemic foci (summarized by Agnew et al. (1993)), as well as in mixed foci (Hairston, 1965; Clarke, 1966; Farooq et al., 1966a; Dennis et al., 1983; Kvalsvig and Schutte, 1986; Robert et al., 1989; De Clercq et al., 1999; Lwambo et al., 1999; El Khoby et al., 2000) have shown similar trends, but only Agnew et al. (1993) explicitly mentioned this. In an early autopsy study, Cheever et al. (1977) reported a more pronounced reduction of *S. haematobium* than *S. mansoni* worm loads with age, which is also in line with our results. The underlying mechanism for this apparently rather common phenomenon is unknown and merits further investigation. Different mechanisms could play a role and are discussed below.

Cheever et al. (1977) showed that *S. haematobium* eggs have a higher tendency to accumulate at the oviposition site than *S. mansoni* eggs. Accumulating *S. haematobium* eggs lead to progressive bladder wall pathology which may subsequently obstruct egg passage into the lumen of the bladder and lead to a reduction of *S. haematobium* egg excretion. Furthermore, differences between *Schistosoma* spp. in age-dependent reduction of fecundity have been suggested, possibly mediated by the host's immune system (Agnew et al., 1996).

Differences in vulnerability to the host's immune response between the two species may also explain the more pronounced reduction of *S. haematobium* compared with *S. mansoni* worm load with age as observed by Cheever et al. One human study indicated that the two species induce different types of humoral immune responses (van Remoortere et al., 2001). The authors showed that *S. haematobium*-infected subjects produced IgM as well as IgG antibodies against specific carbohydrate epitopes, while IgM – which is thought to inhibit protective host immune responses (Butterworth et al., 1987) – dominated in *S. mansoni* infections. Also, animal studies reported vaccination with cercariae to convey a higher degree of protection against *S. haematobium* than against *S. mansoni* infection (Taylor et al., 1973; Webbe et al., 1976; Agnew et al., 1993; Dean et al., 1996). Similarly, animal models provided evidence for differences in 'concomitant immunity': adult *S. mansoni* worms appear to have a greater capacity than *S. haematobium* to elicit an immune response which prevents infection with new worms, while they themselves remain invulnerable to the host's immune defense (Agnew et al., 1993; Terry, 1994).

Omer and Teesdale (1978) suggested that the apparent increased vulnerability of *S. haematobium* compared with *S. mansoni* may be related to the location of the worms in the human blood circulation. The authors suggested that treatment-induced damage to, and dislodgement of, adult *S. haematobium* would result in displacement of the worms within the blood stream from the vesical plexus via the heart to the lungs. Trapped in the capillary beds of the lung alveoli, they would not be able to recover from this damage (i.e. so-called 'irreversible lung-shift'). Damaged adult *S. mansoni* worms, on the other hand, would be carried away from the mesenteric plexus via the portal vein to the liver where they can recover and then return to their oviposition site. Although speculative, similar mechanisms may play a role upon immune damage to adult worms.

The observed differences between age-related *S. mansoni* and *S. haematobium* patterns may also be related to differences in exposure to the two species; a lower cumulative exposure to *S. mansoni* compared with *S. haematobium* could have resulted in a lower protective immune response against the first species (Woolhouse, 1998; Mitchell et al., 2008). However, this scenario is unlikely in this specific area because *S. mansoni* was introduced before *S. haematobium* (Picquet et al., 1996), and current exposure to the first appeared more intense; peak and overall (heavy) infection prevalences were higher for *S. mansoni* at the time of this current study.

This suggests that differences in host-parasite interactions play a more important role than parasite exposure in the observed, more pronounced decline of *S. haematobium* than of *S. mansoni* infection with age. It should be noted however, that most evidence relies on animal models and/or old data. Caution should be used when extrapolating observations from animal models to the human host, particularly with regard to *S. haematobium* for which humans are assumed to be the only natural final host (Cook and Zumla, 2009). More recent human studies are needed to corroborate our age-related observations on mixed *Schistosoma* infections as well as on the proposed underlying mechanisms.

Not only age, but also other risk factors for mixed infection observed in this study were similar to those generally observed for schistosomiasis. Schistosomiasis is characterized by a focal epidemiology (Anderson and May, 1985a). It is therefore not surprising that also for mixed infections, community of residence was identified as an important risk factor. This could be due to differences in water contact behavior, snail distribution, genetic differences and other factors (Robert et al., 1989; Pinot de Moira et al., 2007). Furthermore, women were more at risk of infection than men. Gender differences have been previously observed (Dennis et al., 1983; Robert et al., 1989; El Khoby et al., 2000; Cunin et al., 2003), and have been attributed to hormonal differences (Remoue et al., 2002; Klein, 2004; Escobedo et al., 2005) and differences in

water contact between males and females (Fulford et al., 1996; Mahmoud, 2001; Scott et al., 2003; Sow et al., 2011).

Understanding the exact relation between mixed infection and infection intensity is crucial, as increased egg loads can have important repercussions on the development of morbidity (Gryseels et al., 2006). We found higher *S. mansoni* and *S. haematobium* infection intensities in mixed than in single infections and a positive association between *S. mansoni* and *S. haematobium* infections in these mixed foci. Robert et al. also found higher infection intensities in mixed infections (1989). However, other studies on a larger scale (at county, provincial or district level) reported inconsistent results (Dennis et al., 1983; Ahmed et al., 1996; Booth et al., 1998; Lwambo et al., 1999). Possibly, the relationship between mixed infection and infection intensity varies according to local differences in *S. mansoni* and *S. haematobium* transmission, which may be diluted on a larger scale. As such differences can even occur at a community level (Pinot de Moira et al., 2007), it is important to further investigate the relationship between mixed infection, infection intensity and morbidity in small-scale studies and different endemic settings.

Ectopic eggs were found in 15% of mixed infections, and most of those were *S. mansoni* eggs. Single infection with ectopic egg elimination was uncommon. Ectopic *S. mansoni* egg elimination was associated with *S. mansoni* and *S. haematobium* infection intensity. A 'spilling over' of *S. mansoni* worms and/or eggs towards the urinary bladder may have partly contributed to the elimination of *S. mansoni* eggs via the urine in heavily infected children (Husting, 1965). It has also been proposed that increased portal pressure – due to severe *S. mansoni*-associated hepatic fibrosis – might contribute to this phenomenon (Cook and Jordan, 1970; Cheever et al., 1977). Nevertheless, ectopic *S. mansoni* egg elimination was more strongly associated with *S. haematobium* than with *S. mansoni* infection intensity. Previous studies have consistently found this association (Blair, 1965; Husting, 1965; Ratard et al., 1991; Cunin et al., 2003) which has been attributed to sexual interactions between the two species (Webster et al., 1999). Experimental models have shown that *S. mansoni* and *S. haematobium* can form heterologous male-female pairs. This results in *S. haematobium* males carrying *S. mansoni* females to the vesical plexus. These females will then lay eggs with a *S. mansoni*-like morphology that are passed into the urine (Southgate et al., 1998). In addition, single males can remove homo- or heterologous females from other male worms (Tchuem Tchuente et al., 1995; Pica-Mattoccia et al., 2000; Steinauer, 2009). Male *S. haematobium* appear to be competitively stronger in taking heterologous females away from their male partner than *S. mansoni* (Webster et al., 1999; Cunin et al., 2003). This might explain why *S. mansoni* eggs are more commonly found to be eliminated via the unusual route than *S. haematobium* eggs (Ratard et al., 1991; Ernould et al., 1999a; Cunin et al., 2003);

prevalences of ectopic *S. mansoni* elimination of up to 31% have been reported (Ernould et al., 1999a).

In the present study, ectopically eliminated eggs were categorized as either *S. mansoni* or *S. haematobium.* However, we cannot exclude that some of these may have been genetic hybrids (Huyse et al., 2009), or parthenogenetic eggs, which could not be distinguished from regular *Schistosoma* eggs by microscopy. Experimental studies suggest that ectopic *S. mansoni*-like eggs are likely to be of parthenogenetic origin (Taylor, 1970; Basch and Basch, 1984; Imbert-Establet et al., 1994; Tchuem Tchuente et al., 1994; Jourdane et al., 1995; Khalil et al., 1995; Southgate et al., 1998). To date however, the possibility that *S. mansoni* x *S. haematobium* hybrids exist in nature has not been excluded. It is essential to determine the exact genetic nature and viability of ectopic *Schistosoma* eggs since hybrid species are assumed to be more infective and pathogenic than their parental species (Wright and Ross, 1980; Webster and Southgate, 2003; Huyse et al., 2009).

Initially, the distributions and risk factors for mixed infections did not appear to differ much from those of overall *S. mansoni* or *S. haematobium* infections in these mixed foci. Looking at the data in more detail, however, the decline in infection prevalences and intensities in adults was steeper for *S. haematobium* than for *S. mansoni*, resulting in a decrease in mixed infections and a relative increase in single *S. mansoni* infections over age. These observations are in line with previous studies in humans. Also, animal studies suggested *S. mansoni* to be less vulnerable to the host's age-dependent immune response than *S. haematobium*. Furthermore, a positive association was found between mixed infection, ectopic *S. mansoni* egg elimination and infection intensity of both species, with potentially important consequences for the development of morbidity in co-endemic areas. The significance of these findings should be confirmed by further epidemiological studies at a micro-geographical level, taking host- and parasite-related as well as environmental factors into account.

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