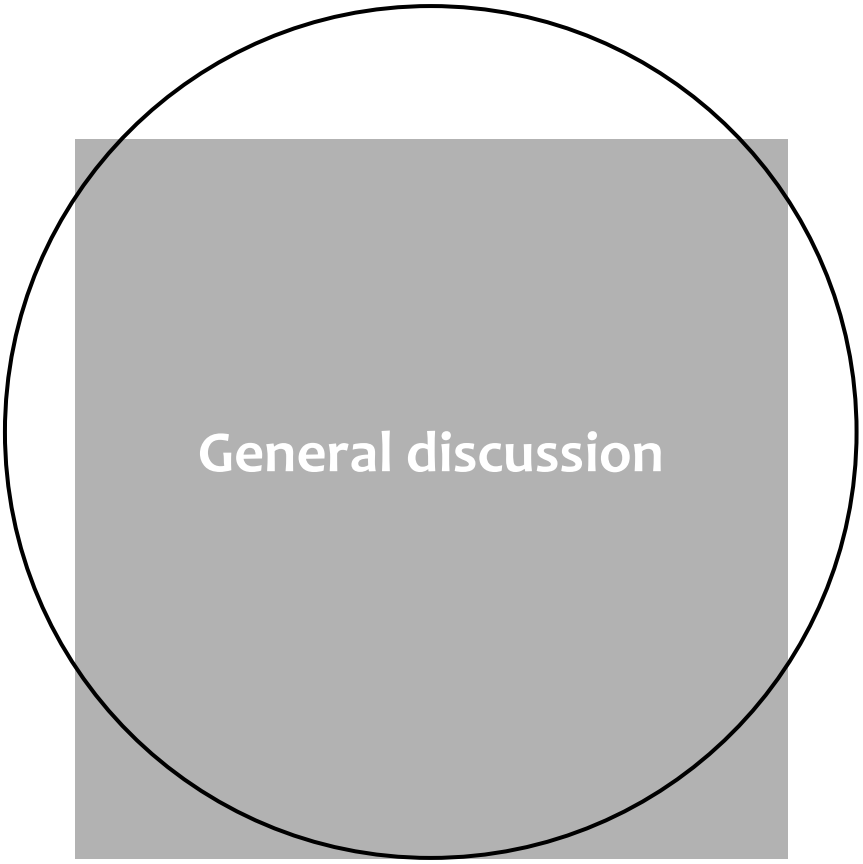
Chapter 7



Thesis outline

Schistosomiasis is one of the most common human parasitic diseases with over 230 million people affected worldwide. The two major species are *Schistosoma mansoni* and *S. haematobium*, which occur together in many African regions (Gryseels et al., 2006). In such co-endemic areas, the two species can interact, either directly or indirectly via the host immune system. This may have important implications for infection and morbidity levels in the communities involved. However, few studies have investigated this. This thesis is one of the first investigations into differences and interactions between the two major human *Schistosoma* species, and their effects on host morbidity.

In the previous chapters, patterns of infection and morbidity were investigated in a detailed way by zooming in into one co-endemic focus in the north of Senegal, Nouk Pomo, and by adopting a multidisciplinary approach including epidemiological, parasitological, clinical, geographical, and immunological investigations. We compared demographic determinants of *S. mansoni* and *S. haematobium,* single and mixed infections, *S. mansoni* and *S. haematobium* morbidity, as well as the effect of mixed and single infections on morbidity (Chapter 2 and 3). The micro-geographical distribution of *S. mansoni* and *S. haematobium* infection and morbidity, and that of single and mixed infections were studied (Chapter 4), and immunological patterns of *S. mansoni* and *S. haematobium* infection were compared in order to gain more insight into the mechanisms that may underlie epidemiological observations (Chapter 5 and 6).

In this chapter, we will integrate the findings of the different chapters and take them one step further. We present a comprehensive analysis of factors related to mixed infections and morbidity, and provide an in-depth analysis of age-related infection patterns and immune responses in (co-)endemic situations. The relevance of the findings in this thesis for schistosomiasis control and elimination will be highlighted, and we will conclude with directions for further research.

Mixed infections and morbidity

In Africa, many people are at risk of infection with both *S. mansoni* and *S. haematobium* (Gryseels et al., 2006). It is therefore important to understand, whether and how such mixed *Schistosoma* infections may alter the risk of developing chronic morbidity. This is a challenging research question because chronic morbidity due to schistosome infection generally develops over the course of many years, and it is difficult to follow a population for such a long period of time, if only for ethical reasons. The transversal, multidisciplinary studies in this thesis nonetheless provide some important insights into the possible determinants of morbidity in populations co-endemic for *S. mansoni* and *S. haematobium*. Here, we will further discuss and link the possible effects of

heterologous worm pairing, infection intensity, and egg-induced immune responses on host morbidity in mixed infections.

Heterologous worm pairing

We found that people with the heaviest infections often had ectopic *S. mansoni* eggs in urine. A 'spilling over' of the surplus of *S. mansoni* worms and/or eggs towards the urinary bladder may have contributed to this phenomenon. However, ectopic *S. mansoni* eggs were also observed in a minority of people without *S. mansoni* eggs in feces, and in whom 'spilling over' was therefore less likely. In Chapter 3, this specific subgroup of people with both *S. haematobium* and ectopic *S. mansoni* eggs in urine, but without *S. mansoni* eggs in feces (n=6), tended to be less at risk of *S. haematobium*-specific urinary tract morbidity than those with other *S. haematobium* infections. It was argued that this may have been due to direct interactions between the two *Schistosoma* species. *Schistosoma haematobium* males probably outnumber and/or outcompete *S. mansoni* males, and are able to pair with heterologous *S. mansoni* females. In such heterologous male-female pairs, the *S. haematobium* male determines the oviposition site (region of the urinary tract) and the *S. mansoni* female the egg morphology (lateral spine). Perhaps, less (pathogenic) eggs are produced upon heterologous pairing, or these eggs may be more likely to deviate to other sites. While this thesis was in preparation, a study in Kenyan school children observed very similar patterns, which may also be explained by heterologous worm pairing. Although that study did not take ectopic egg elimination into account, children with mixed infections were less at risk for urinary tract morbidity than those with single *S. haematobium* infections (Gouvras et al., 2013). Moreover, Huyse et al. (2013) discovered *S. mansoni* x *S. haematobium* hybrid eggs in children living in the same area as our study population. Like ectopic eggs, these hybrids were observed more frequently in urine than in feces. It is therefore tempting to speculate that the ectopic *S. mansoni* eggs observed in this thesis may have included genetic hybrids.

Heterologous worm pairing in the intestinal tract on the other hand, is less likely to occur as *S. haematobium* males have been shown to be competitively stronger than *S. mansoni* males (Webster et al., 1999; Cunin et al., 2003). Consistent with this, ectopic *S. haematobium* egg elimination in feces was rare in the present study (Chapter 2).

Infection intensity

In Chapter 2, we found higher infection intensities of *S. mansoni* as well as *S. haematobium* in mixed as opposed to single infections. As far as we are aware, this is the only study that has been performed on such a small geographical scale. Studies that were performed on a larger geographical scale reported inconsistent results, which could have been due to local variations in transmission dynamics of the two

species. Robert et al. (1989) for example, reported comparable infection intensities in mixed and single *S. mansoni* infections but elevated intensities in mixed as compared with single *S. haematobium* infections in one population living in different communities on the banks of the Lagdo Lake in northern Cameroon. Preliminary results from our group in another village - Pakh - in the Lac de Guiers area, indicated significantly elevated levels of *S. haematobium* but reduced levels of *S. mansoni* in mixed as compared with single infections. As it is unknown which factors underlie these differences, it remains unclear to which extent the association between mixed infection and infection intensity observed in this thesis can be extrapolated to other populations.

The association between *S. haematobium* infection intensity and *S. haematobium*-specific urinary tract morbidity appeared stronger than that between *S. mansoni* infection intensity and *S. mansoni*-specific hepatic fibrosis. In Chapter 3, a direct association was found between active infection and urinary tract morbidity, but not hepatic fibrosis. Similar patterns were found in a *S. mansoni* and *S. haematobium* co-endemic focus in Egypt (Farooq et al., 1966b). The micro-geographical patterns in Chapter 4 strongly suggested an association between the level of *S. mansoni* infection intensity earlier in life and the severity of hepatic fibrosis later on in adult life. The difference in the delay between infection and the onset of morbidity may be due to interspecies differences in egg pathogenicity (see 'Egg-induced immune responses' below), or to differences between the anatomical context of the liver and urinary tract.

The same micro-geographical analyses showed that severe hepatic fibrosis clustered close to the central water contact site, in the same area where children showed the heaviest infections of the community. Preliminary analyses did not indicate any evidence of spatial heterogeneity in egg-induced cytokine responses, nor in any of the other adaptive or innate cytokine responses measured (data not shown). This strongly suggests that, on the community level, (cumulative) exposure to *Schistosoma* eggs may be more important in determining the risk of developing morbidity than individual host immune responses. Yet, it remains to be investigated whether there may be more subtle effects of cytokine responses on host morbidity, or whether immune parameters other than the cytokine responses measured here may provide a better indicator for who is likely to develop morbidity and who not, and how this relates to mixed infections.

Egg-induced immune responses

As explained above, people who excreted both *S. haematobium* and ectopic *S. mansoni* eggs in urine, but no *S. mansoni* eggs in feces tended to be less at risk of bladder morbidity. Due to an insufficient number of people with this type of infection in the immunological study, we could not draw any conclusions on the immune responses in

these subjects. Hence, it remains to be determined whether any protective effect of heterologous worm pairing might be immune-mediated. In general, people with mixed infections produced lower levels of cytokines than those with single infections, but with the approach used it was not possible to determine whether this was due to co-infection per se or due to elevated infection intensity, or to other potential confounders. The similarity of *S. mansoni*- and *S. haematobium*-induced cytokine profiles rather suggested that co-infection with *S. mansoni* is unlikely to have skewed immune response against *S. haematobium* eggs towards a potentially more or less pathogenic phenotype, and vice versa.

Nonetheless, it was shown that the magnitude of these responses were higher upon stimulation with *S. haematobium* as compared to *S. mansoni* (egg) antigens, suggesting that *S. haematobium* might induce stronger and therefore potentially more pathogenic immune responses than *S. mansoni*. Indeed, *S. haematobium*-specific morbidity was more common than *S. mansoni*-specific morbidity (Chapter 3), and age distributions indicated that the delay of onset of morbidity may be shorter for *S. haematobium* than for *S. mansoni* (Figure 3.1). Moreover, people without putative hybrid *S. mansoni* x *S. haematobium* eggs were more at risk for *S. haematobium*-specific morbidity than those with these ectopic *S. mansoni*(-like) eggs (Table 3.6).

As discussed in Chapter 5, this hypothesis is in line with findings of Van Remoortere et al. (2001). These authors showed that *S. mansoni* induces mainly IgM – which is thought to inhibit protective host immune responses (Butterworth et al., 1987) – while *S. haematobium* induces both IgM and IgG antibodies against shared carbohydrate

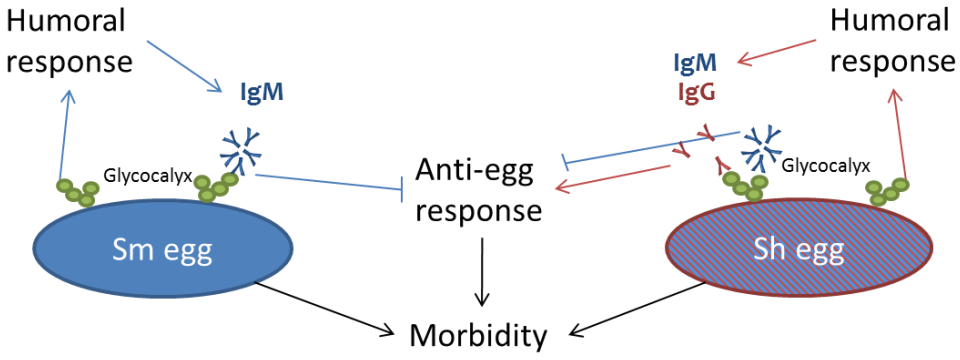


Figure 7.1. Hypothesized effects of *S. mansoni* and *S. haematobium* infection on host morbidity.

Van Remoortere et al. (2001) demonstrated that the antibodies to specific glycan epitopes from *S. mansoni* life stages induce more IgM and less IgG than those from *S. haematobium* life stages. The inhibitory effect of IgM might reduce anti-egg responses of the host immune system and thereby the development of host morbidity in *S. mansoni* co-infections, while increased IgG levels in *S. haematobium* co-infections may have the opposite effect.

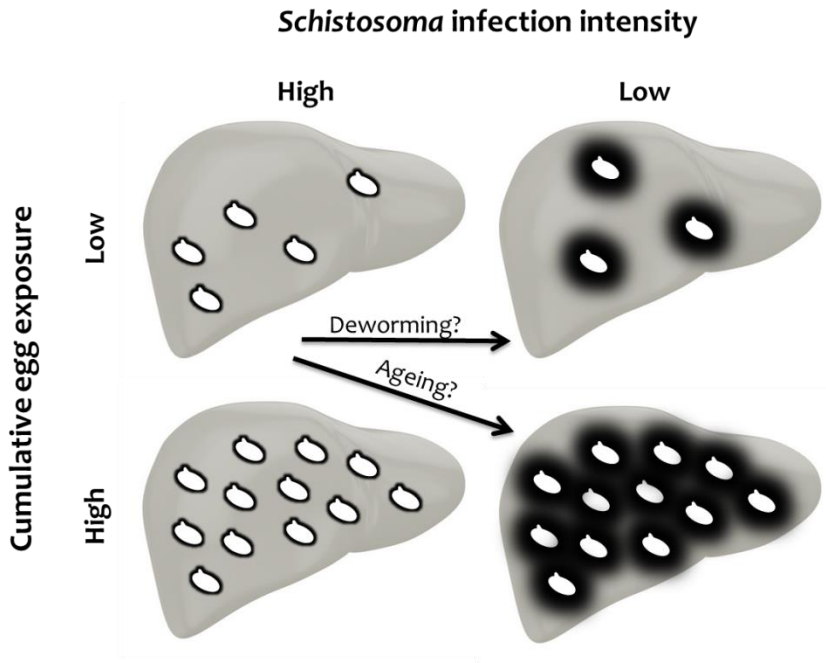


Figure 7.2. *Schistosoma* infection intensity, cumulative egg exposure, egg-induced immune responses, and morbidity in schistosomiasis.

Chronic hepatic schistosomiasis is used here as an example to demonstrate the relationship between *Schistosoma* infection intensity, cumulative exposure to *Schistosoma* eggs, and local immune responses to tissue-trapped eggs which may altogether lead to morbidity. The number of eggs in the liver indicates the number of trapped eggs or lesions with immuno-reactive remnants. Sizes of the black areas surrounding the eggs indicate the relative strength of the immune reactions in individual tissue lesions. We hypothesize that in the endemic setting, as people age, they will move from low to high cumulative egg exposure and from high to low infection intensities, and thus have enhanced immune responses against individual eggs (bottom right). After deworming, *Schistosoma* infection intensities decrease and immune responses against tissue-trapped eggs may become more pathogenic.

epitopes. IgM responses against trapped eggs might protect *S. mansoni*-infected individuals from developing morbidity, while increased IgG levels might predispose *S. haematobium*-infected individuals to more severe morbidity. Interestingly, IgG levels against the same carbohydrate epitopes were shown to be even higher in *S. japonicum* infections (Van Remoortere et al., 2001), which are associated with a more rapid onset of chronic morbidity and with more severe morbidity than *S. haematobium* (Jordan et al., 1993). We therefore cautiously hypothesize that *S. mansoni*-induced glycan-specific IgM may inhibit anti-egg responses and contribute to reduced levels of urinary tract morbidity in mixed as compared to single *S. haematobium* infections. Conversely, *S.*

haematobium-induced IgG might be hypothesized to enhance anti-egg responses and lead to increased levels of hepatic morbidity in mixed as compared to single *S. mansoni* infection, as illustrated in Figure 7.1.

It should be taken into account that the relationship between active infection and morbidity may not be as straightforward as is generally assumed. Chapter 5 suggested that heavily infected individuals have low immune responses to the eggs trapped in host tissues, while those with lighter infections have stronger immune responses to individual eggs. People with light infections are generally older, and are likely to have accumulated more *Schistosoma* eggs than younger individuals with heavy infections. This may imply that the pathogenicity of individual, trapped eggs might increase as the host ages. In other words, high cumulative exposure and reduced levels of infection might have synergistic effects on the development of chronic morbidity in schistosomiasis in older age. As explained in Figure 7.2, this may also imply that (temporary) worm elimination upon praziquantel treatment may lead to exacerbation of egg-induced morbidity.

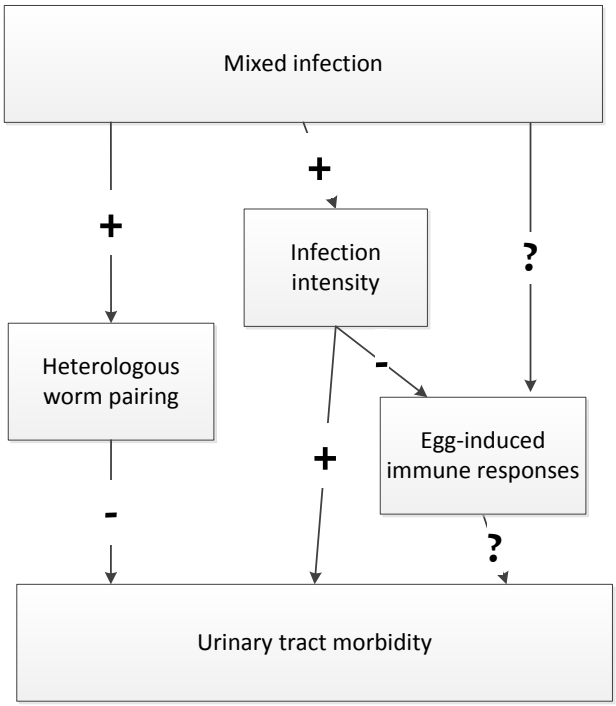


Figure 7.3. Overview of the factors that were studied and the relationships that were found with mixed infections and *S. haematobium*-specific urinary tract morbidity.

Net effect on morbidity

Figure 7.3 summarizes how heterologous worm pairing, infection intensity, and egg-induced immune responses may together (partly) determine the net effect of mixed infection on host morbidity, with *S. haematobium*-specific urinary tract morbidity as an example. Briefly, heterologous worm pairing might lead to less (pathogenic) *Schistosoma* eggs in the urinary tract, than pure *S. haematobium* infections. On the other hand, infection intensities were found to be higher in mixed *Schistosoma* infections, but it remains to be investigated whether a causal relationship exists between co-infection and infection intensity. Increased infection intensities lead to higher cumulative exposure of host tissues to *Schistosoma* eggs and thus to higher morbidity levels. We could not identify any interspecies differences in the induction of systemic cytokine profiles - i.e. regulatory, Th1, or Th2 phenotypes – but other immune parameters that were not measured (e.g. humoral immune responses) may still explain the observed differences in morbidity between single and mixed *Schistosoma* infections.

Age, *Schistosoma* infection and Th1 immunity

In Chapter 5, multiple cytokine responses to each of four *Schistosoma* antigens tested were visualized using nonmetric multidimensional scaling (nMDS). This resulted in four cytokine profiles. Associations between these cytokine profiles and *Schistosoma* infection were subsequently investigated. We found that both *S. mansoni* and *S. haematobium* infection were positively associated with antigen-induced Th2 and negatively with inflammatory/Th1 cytokines. This corresponds with results from earlier studies in either *S. mansoni* or *S. haematobium* mono-endemic areas (e.g. that of Joseph et al., 2004). These immuno-epidemiological patterns suggest that Th1 rather than Th2 responses may protect against infection, and confirms previous observations in mouse models (Wilson and Coulson, 2009). Moreover, worm antigen-induced Th1 responses consistently showed a linear increase with age (data not shown), suggesting that such protective immunity may be acquired with age - building up over time through cumulative exposure to schistosomes.

Besides *Schistosoma* antigen-induced cytokine profiles, we also studied cytokine profiles that are not specific for schistosomes, i.e. those induced by the T-cell mitogen phyto-hemagglutinin (PHA). Age as well as infection trends were very similar to those induced by *Schistosoma* worm antigen-induced cytokine profiles. nMDS furthermore showed that all PHA-induced cytokine responses were positively associated with *Schistosoma* worm antigen-induced Th1 cytokines ($p<0.01$; data not shown).

To investigate whether age may have confounded the association between cytokine profiles and infection intensity and/or vice versa, we used redundancy analysis (RDA) (Borcard et al., 2011). This is a statistical technique that is commonly used in

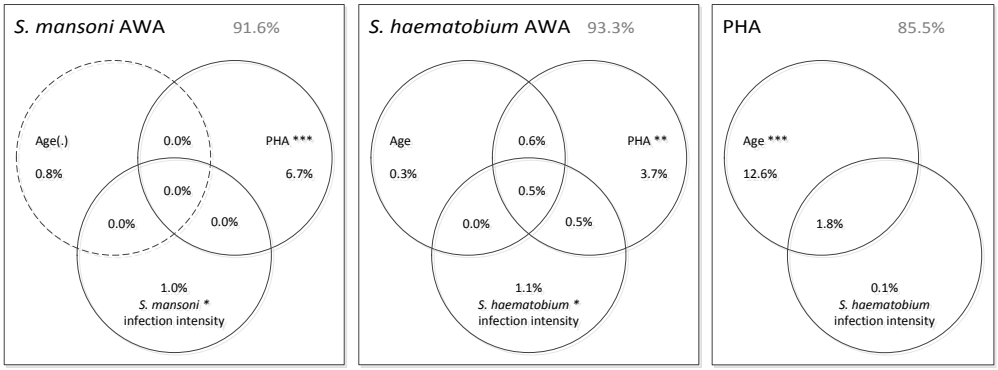


Figure 7.4. Variation partitioning by multivariable redundancy analysis: worm and mitogen-induced cytokine profiles, age and infection.

Venn diagrams indicate the percentages variance in antigen-induced cytokine profiles that could be explained by different explanatory variables (represented by circles) in multivariable RDA models and the percentages of variance that remained unexplained (outside circles) (n=199). Prior to RDA ('rda' function of Vegan package in R), net cytokine responses were log-transformed and standardized as for nMDS (see Chapter 5). Explanatory variables (age, *S. mansoni* and *S. haematobium* infection intensity, and for *Schistosoma* adult worm antigen (AWA)-induced cytokine profiles: PHA-induced cytokine profiles) were included in RDA models if they were significantly associated with respective 3D nMDS cytokine profiles. Except for the association between age and *S. mansoni* AWA-induced profiles (dashed circle), all associations were confirmed in simple RDA (continuous circles). The diagrams show that the association between infection intensity and AWA-induced cytokine responses remained in multivariable RDA. Also, the association between PHA- and AWA-induced cytokine profiles remained statistically significant in multivariable RDA. On the other hand, PHA responses were only associated with age - not with *Schistosoma* infection - and the significant effect of age on AWA-induced responses, was lost when PHA-responses were taken into account in the multivariable model. Asterisks indicate significance levels of the independent effects in full RDA models: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

ecology – but to our knowledge, never in biomedical sciences – and that combines linear regression with principal component analysis. Also, RDA was used to investigate the effect of unspecific, PHA-induced cytokine response profiles on *Schistosoma* worm-specific responses.

RDA showed a very specific and independent immunoregulatory/Th2-skewing effect of infection on *Schistosoma* worm-induced cytokine profiles. In addition, RDA indicated an infection-independent effect of age on general (i.e. nonspecific) adaptive immune responses as measured by PHA-induced cytokine profiles (Figure 7.4). Indeed, ageing itself is associated with dramatic changes in T cell function (Gardner and Murasko,

2002), and a Ghanaian study observed a similar age-associated increase in innate Th1 responses (May et al., 2009). The fact that, under adverse living conditions, subjects with a pro-inflammatory genetic predisposition are more likely to survive up to old age than those without might contribute to these observations (Van Bodegom et al., 2007; Kuningas et al., 2009). However, age-related increases in inflammation, termed 'inflamm-ageing', have also been described in more affluent populations (e.g. Franceschi et al., 2000).

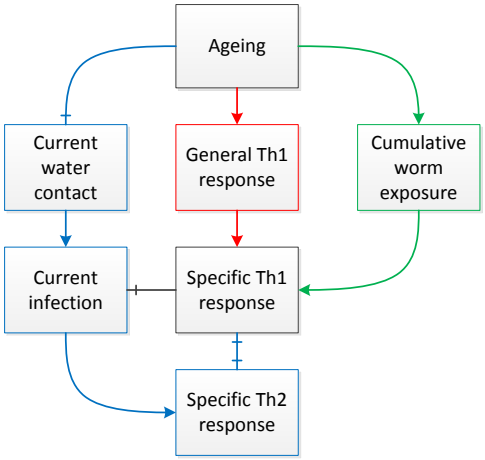


Figure 7.5. Conceptual framework of the hypothesized triangular relationship between age, Th1 responses, and *Schistosoma* infection.

Age-related infection and immunological patterns, as well as observations in Chapter 5 suggested that worm-specific Th1 responses might play a central role in immunological protection against infection. Arrowheads indicate positive and cross lines negative associations.

Disentangling the triangle

As illustrated in Figure 7.5, we hypothesize three different effects of ageing on *Schistosoma* infection and *Schistosoma*–specific Th1 immune responses. These may underlie *Schistosoma* infection-age infection curves in endemic areas:

- Due to differences in water contact behavior, adults are assumed to be less exposed to *Schistosoma* infection than children. This, or other factors that were not measured (e.g. age-related hormonal, metabolic, or dermatological changes) would lead to reduced infection levels across age groups, and the absence of worms would give rise to a relative decrease in Th2 and increase in Th1 responses (blue chain of events).
- In an endemic setting, exposure to *Schistosoma* antigens builds up as people age. This may give rise to a gradual increase in protective immunity and our observations indicate that such adaptive responses are most likely of the Th1 phenotype. These responses would in turn lead to a reduction of infection with age (green chain).

- Factors inherent to ageing itself may lead to a progressive increase in general pro-inflammatory/Th1 responses, or there may be selective survival of people with a genetic predisposition to produce pro-inflammatory/Th1 responses. Consequently, worm-specific immune responses would be skewed towards the Th1 phenotype in older individuals (red chain). If such Th1-type responses are indeed protective, this may lead to reduction of infection levels over age.

Relevance for schistosomiasis control strategies

A thorough knowledge of transmission of infection and disease etiology is key for successful schistosomiasis control. This thesis described the epidemiological patterns of *S. mansoni* and *S. haematobium* (co-)infection and disease at the smallest scale, i.e. at the micro-geographical level where disease transmission actually occurs. Strong spatial clustering at this level once more confirmed that such a fine geospatial resolution is essential to understand this complex disease and hence to develop sustainable control and elimination strategies. Based on our micro-epidemiological approach, we identified some opportunities and recommendations that should be taken into account to improve current strategies.

Mass drug administration

Currently, control strategies are based on periodic mass drug administration (MDA) with praziquantel. MDA aims to reduce infection levels early in life with the aim of reducing the development of chronic morbidity later in life. As explained above, our cross-sectional findings indeed suggest that cumulative exposure to infection is the main driver of severe morbidity (see 'Mixed infections and morbidity: Infection intensity'), and as such, support this basis.

The WHO recommends national control programs for schistosomiasis to target (mainly) school-age children and other high-risk groups (Table 7.1). Observations from this thesis suggest that optimizing current treatment allocation strategies may improve the effectiveness of MDA. Recently, the WHO has recognized that preschool-age children may be at a similar risk of schistosome infection and morbidity as their school-age siblings, and that they should be targeted as well (WHO, 2013). However, our results indicate that not only preschool-age but also children older than 14 years and young adults continue to be infected. For *S. mansoni*, infection prevalences and intensities only peaked after school-age, i.e. in 15-19-year olds (Chapter 2). These older age groups are thus likely to contaminate the environment, and to develop morbidity over time. Instead of school-(age-)based MDA, we thus recommend community-wide MDA, not only in high-risk areas, but also in areas that are at moderate and low risk (at least for *S. mansoni*).

Table 7.1. Treatment strategy for schistosomiasis recommended by the WHO (2013).

Category	Baseline prevalence among school-age children	Action to be taken	
High-risk community	≥50% by parasitological methods [a] (*S. mansoni* and *S. haematobium*) or ≥30% by questionnaire for history of hematuria	Treat all school-age children (enrolled and not enrolled) once a year	Also treat adults considered to be at risk [b] (from special groups to entire communities living in endemic areas)
Moderate-risk community	10-50% by parasitological methods (*S. mansoni* and *S. haematobium*) or <30% by questionnaire for history of hematuria	Treat all school-age children (enrolled and not enrolled) once every 2 years	Also treat adults considered to be at risk [b] (special groups only)
Low-risk community	<10% by parasitological methods (*S. mansoni* and *S. haematobium*)	Treat all school-age children (enrolled and not enrolled) twice during their primary schooling age (e.g. once on entry and once on exit)	Praziquantel should be available in dispensaries and clinics for treatment of suspected cases

[a] For *S. haematobium*, detection of hematuria by dipstick tests gives results equivalent to those determined by urine filtration.

[b] From special groups (pregnant and lactating women, groups with occupation involving contact with infested water such as fishermen, farmers, irrigation workers or women in their domestic tasks) to entire communities living in endemic areas

Current MDA allocation strategies do not reflect the spatial epidemiology of schistosomiasis infection and morbidity. In Chapter 4, we observed a highly focal distribution of both infection and associated disease on the micro-geographical level. We found that people living within 100m of the major water contact site had a more than six-fold higher risk of developing severe hepatic fibrosis than those who lived farther away. Nevertheless, the WHO recommends collecting baseline infection data based on parasitological surveys in sentinel sites that are assumed to be representative for a large so-called 'homogeneous ecological zone' (WHO, 2011). The recommended treatment strategy for this entire zone, i.e. 200,000-300,000 targeted children subsequently depends on the observed prevalence in 50 children from one randomly selected sentinel school. Obviously, such a uniform approach is prone to fail in reaching (all) those individuals who are most in need of treatment, and will result in unnecessary treatment of uninfected people. A substantial increase in the number of sentinel sites may help to overcome this problem, but would be incompatible with the logistical and economic advantages underlying current MDA-based control strategies (Molyneux et al., 2005). Novel ways are therefore urgently needed to enable a more targeted allocation of control interventions in *Schistosoma*-endemic areas. Based on the results of this thesis, we propose to use environmental factors such as the presence of infested water bodies and the geographical distance to these *Schistosoma* 'epicenters'

for treatment allocation, rather than parasitological data from randomly selected schoolchildren.

Other findings in this thesis suggested that removal of the causative agent might not simply lead to a proportional reduction of morbidity, especially in co-endemic areas. An unexpected effect of mixed *S. mansoni* and *S. haematobium* infections on *S. haematobium*-specific urinary tract morbidity was observed. The presence of *S. mansoni* appeared to protect against this form of morbidity in some cases. Also, immune responses in individuals with low levels of infection appeared more pathogenic than in individuals with higher infection levels. In addition, other studies in *S. mansoni* and *S. haematobium* co-endemic areas have shown an increase rather than a decrease of *S. mansoni* infection after deworming (Omer and Teesdale, 1978; De Clercq et al., 1999; Ernould et al., 1999a; Koukounari et al., 2010; Webster et al., 2013; Gouvras et al., 2013). Hence, periodic MDA and subsequent changes in immunological and infection equilibria may (in some cases) exacerbate the development of chronic morbidity instead of reducing it (Daffalla and Fenwick, 1982).

Elimination

Gaining and sustaining control of schistosomiasis and possibly achieving local elimination are the year 2020 targets set by the WHO (Knopp et al., 2013). When moving from control to elimination, active surveillance and detection of increasingly low-transmission areas is needed, but proven very difficult. Today, it is increasingly being recognized that malaria transmission becomes more and more focal as malaria prevalences are reduced (Cotter et al., 2013). As similar phenomena may occur in a post-control setting for schistosomiasis, strategies need to be developed with a sufficiently high geospatial resolution to detect even the smallest schistosomiasis hotspots. Because it will not be cost-effective to routinely collect parasitological data from human populations in a post-control setting, new ways of detecting schistosomiasis should be sought, e.g. through environmental sampling (see also 'Mass drug administration'). In addition, it is expected that MDA alone cannot break the *Schistosoma* life cycle and that complementary interventions will have to be put in place for schistosomiasis elimination, such as the provision of clean water and improved sanitation, snail control, and behaviour change (Sturrock, 1989; King, 2009; Gray et al., 2010; Rollinson et al., 2013; Freeman et al., 2013). Multidisciplinary studies on the micro-geographical level such as the ones described in this thesis will help to get much needed insights into local transmission dynamics of *S. mansoni* and *S. haematobium* and hence to move from control towards elimination.

Conclusions and directions for further research

More studies are needed to investigate to which extent the observations in this thesis can be extrapolated over time and to other communities. Future studies may build on the findings of this thesis, on the hypotheses that were generated, and on the novel methodologies used. Here, we will discuss some main directions for further schistosomiasis research.

Health impact of mixed *Schistosoma* infections

Helminth co-infections and mixed *Schistosoma* infections are the rule rather than the exception. Nonetheless, co-infection and mixed infection remain an understudied element of human medicine. This thesis provides a significant addition to the limited number of studies on mixed *Schistosoma* infections in humans. We demonstrated that on the small scale, mixed *Schistosoma* infections are significantly associated with increased infection intensities. However, different relationships between mixed infection and infection intensity have been found in other study populations. It is important to understand which context-specific factors impact on the association between mixed infection and infection intensity, and which causal mechanisms underlie this association. For example, it remains to be investigated whether previous and/or concomitant infection with *S. mansoni* might alter host resistance to infection with *S. haematobium*, or whether it may alter *S. haematobium* egg production (or vice versa). Secondly, we found that mixed infections affect the host's health differently than single infections (regardless of infection intensity). We hypothesized that this may be due to immunological differences between *S. mansoni* and *S. haematobium*, and also to heterologous worm pairing. Further studies are necessary to determine whether *S. haematobium* eggs are more immunogenic than those of *S. mansoni*, and to investigate the different features of anti-egg immune responses into more detail. Also, parasitological factors such as infection intensity, ectopic egg elimination, heterologous worm pairing, and hybridization between species should be studied in more detail to answer questions such as: What is the genetic nature of ectopically eliminated *Schistosoma* eggs? What are the morphological and immunological characteristics of *S. mansoni* x *S. haematobium* hybrid eggs? At the same time, confounding factors such as age and micro-geographical processes need to be taken into account. Only such a multidisciplinary approach can unravel the very complex mechanisms that determine the eventual effect of *Schistosoma* co-endemicity and mixed infections on the host's health.

Praziquantel treatment

Mixed *Schistosoma* infections may not only impact on the host's health, but also on the effect of praziquantel treatment. Unfortunately however, little attention is currently

paid to the possibility that concomitant infections in co-endemic populations may reduce drug effectiveness and increase the risk of side effects (Buck et al., 1978). In addition, chemotherapeutical removal of one or more pathogens may clear the way for invasion of other pathogens that may lead to worse health outcomes (Daffalla and Fenwick, 1982). One major question that was not addressed in this thesis was how praziquantel treatment impacts on *Schistosoma* co-infection and morbidity levels. Nonetheless, some of our observations suggested that it might indeed have adverse health effects (e.g. *S. mansoni* infections appeared to protect against *S. haematobium*-specific bladder morbidity, so elimination of *S. mansoni* might enhance this bladder morbidity). Given that praziquantel treatment may have unwanted effects in polyparasitism, and the recent scale-up of MDA programs, closer monitoring and investigations into both beneficial and adverse health effects of MDA with praziquantel are urgently warranted, especially in co-endemic areas (Humphries et al., 2012). Also, more evidence is needed from comprehensive, longitudinal studies to assess the effectiveness of MDA in terms of morbidity reduction, on the short as well as on the long term, and to improve WHO guidelines.

Focal geographic distribution

This thesis contains one of the few studies on the micro-geographical distribution of *Schistosoma* infection and the first on the micro-geographical distribution of associated morbidity. Significant spatial clustering of *Schistosoma* infection was observed even within one community, and *S. mansoni* and *S. haematobium* infection hotspots were found in different sections of the community. Future studies should investigate the drivers of this spatial clustering. It would be important to investigate whether similar divergent distributions of *S. mansoni* and *S. haematobium* also occur in other communities, and if so: what causes this divergence?

In Chapter 2, we found that *S. mansoni* and *S. haematobium* clustered together in the same individuals, while in Chapter 4, geospatial analyses revealed that the two infections clustered in separate parts of the community. These contrasting patterns on the individual and micro-geographical level seem to point to human factors (e.g. individual water contact behavior and/or immunological factors) as an explanation for the co-occurrence of *S. mansoni* and *S. haematobium* on the individual level, rather than micro-geographical factors such as co-exposure to both species in the same water contact site.

Not only *Schistosoma* infection but also associated chronic morbidity showed a very focal geographical distribution on the micro-geographical level, and people living within the close vicinity of the major water contact site of the community were disproportionally affected. This suggests that cumulative exposure to schistosomes may be the main driver of chronic morbidity on the community level, and warrants

further research into the role of this factor in the etiology of chronic *Schistosoma*-specific disease. In order to estimate the effect of cumulative exposure relative to other etiologic factors such as co-infection, host immunology and host genetics, a multidisciplinary approach will be key in unravelling the complexities of disease etiology.

Parasite immunology and vaccine development

Immunological mechanisms undoubtedly underlie epidemiological patterns of infection and disease. Yet, it remains to be investigated to which extent they explain the notoriously heterogenic distribution of schistosomiasis. Further studies should also assess the role of innate immunity in schistosomiasis. Also, the role of host immunological processes in polyparasitism is still unclear. Moreover, longstanding questions remain: To which extent do we develop protective immunity against schistosomes? What is the immunological phenotype of a protective response? Do acquired immune responses drive the typically convex age-infection curve? Further studies are needed to confirm the age-related inflammatory/Th1 shift observed in this thesis, to elucidate the exact biological mechanisms that may drive this shift, and to assess whether it may confer immunological protection against schistosomes in older age. The eventual success of the candidate schistosomiasis vaccines that are currently being tested (Kupferschmidt, 2013), critically depends on the true balance between the different possible underlying mechanisms of the age-infection curve, and whether or not adaptive immune responses are able to reduce levels of infection. Hence, such studies will give crucial insights for vaccine development.

Value of multidisciplinary research

Adding a geospatial dimension to classical immuno-epidemiological investigations proved particularly valuable to increase our understanding of schistosomiasis on the small scale. We demonstrated for example, how spatial analyses may be used as a 'workaround' to study longitudinal relationships, in a cross-sectional study (Chapter 4). This cannot be achieved through classical epidemiological methods. Also, the application of ecological techniques has proven very useful in studying the complexity of immunological responses (Chapter 5). In this way, this thesis provides a novel, more holistic approach to understanding schistosomiasis.

Taking into account the occurrence of co-infections, and a wider adoption of multidisciplinary research is likely to lead to more novel insights and speed up the snail pace at which our knowledge of schistosomiasis is advancing today. Such knowledge is key to rationalizing and optimizing current schistosomiasis control (and possibly future elimination) strategies, particularly in co-endemic areas.