CHAPTER 2

Multiparasitism: A Neglected Reality on Global, Regional and Local Scale

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Contents

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Abstract

This review focuses on the issue of multiparasitism, with a special emphasis on its characteristics, its extent in eastern Asia and its significance for infectious disease control. Multiparasitism is pervasive among socially and economically disadvantaged or marginalised communities, particularly in tropical and subtropical areas. Intestinal parasites are the most numerous group, but an array of parasites is located elsewhere than in the human gastrointestinal tract. Although multiparasitism has been recognised for decades, in-depth studies are rare, and its public health and economic implications have yet to be fully elucidated. The assessment of multiparasitism is hampered by a lack of sensitive broad-spectrum diagnostic tools and the need to collect multiple biological samples for detailed appraisal. Non-specific symptoms and mainly subtle effects complicate the appreciation of its influence on cognitive and physical development, health, economic productivity and general well-being. Multiparasitism has been reported from virtually every eastern Asian country, and studies regarding the extent of multiparasitism and its effects on child health have been implemented in the region. However, new research is needed, as no comprehensive evaluations of multiparasitism in eastern Asia could be identified. Two case studies pertaining to multiparasitism at the local and regional scale are presented. Multiparasitism was rampant in an ethnic minority village in southern People's Republic of China where the challenges associated with its thorough evaluation are illustrated. The results from a cross-sectional survey covering 35 villages highlight the significance of its evaluation for the design of locally adapted and sustainable parasite control and poverty alleviation programmes. We conclude by listing a set of research needs for future investigations.

2.1. INTRODUCTION

Multiparasitism, also known as polyparasitism, can be defined as the concurrent infestation of a single host individual with two or more parasite species. By convention, parasites include members of the eukaryotic protozoa (e.g. flagellates, amoebae, *Sporozoa*, *Coccidia* and *Microspora*; Cox, 2002; Farthing, 2006; Schuster and Chiodini, 2001), helminths (e.g. nematodes, trematodes and cestodes; Bethony et al., 2006; García et al., 2007; Gryseels et al., 2006; Keiser and Utzinger, 2004) and arthropods (e.g. mites, lice and flea; Feldmeier and Heukelbach, 2009; Heukelbach and Feldmeier, 2004). Some studies on multiparasitism in humans also consider bacteria, fungi and viruses (Petney and Andrews, 1998). In this review, the latter organisms will not be considered, since they fall outside traditional parasitology.

The most prevalent human parasites are the common soil-transmitted helminths (STHs; *Ascaris lumbricoides*, *Trichuris trichiura* and the two hookworm species *Ancylostoma duodenale* and *Necator americanus*), *Plasmodium* spp., *Schistosoma* spp., lymph-dwelling filariae, *Pediculus* spp. and *Sarcoptes scabiei*, each allegedly infecting between 100 and 800 million individuals, many of whom are co-infected with two or more of these parasites concurrently (Feldmeier and Heukelbach, 2009; Hotez et al., 2007). Dozens of further common parasites with high incidence rates or considerable prevalence plague humans (Ashford, 1991), for example a host of different intestinal protozoa, such as *Entamoeba histolytica* and *Giardia intestinalis*, members of the genera *Leishmania*, *Typanosoma*, *Taenia*, *Trichinella*, *Strongyloides* and *Echinococcus*, a diversity of trematodes dwelling in the hepato-biliary, respiratory and digestive systems, *Tunga penetrans*, among others. Some of them infect sizable fractions of local populations or occur across many countries and thus are of considerable public health and economic relevance. Most described human parasite species, however, are rarely diagnosed in humans and are of clinical rather than public health importance.

The objective of this chapter is to review the current knowledge regarding multiparasitism in humans, placing emphasis on diagnostic challenges, the public health significance of multiple parasite infections and the extent of multiparasitism in eastern Asia. Two case studies from Yunnan province, southwest People's Republic of China (P.R. China), are presented—one focusing on a single ethnic minority village and the other exploring spatial heterogeneities across an entire county. These studies underscore the scope and limitations associated with the assessment of human multiparasitism. Finally, a number of research needs identified in the literature and through our own investigations are offered for consideration. It is hoped that new research will further our understanding of multiparasitism and its surveillance, prevention, control and eventual elimination.

2.2. MULTIPARASITISM: PERVASIVE AND YET OFTEN NEGLECTED

2.2.1. Extent of multiparasitism

The concurrent infestation of an individual with multiple parasites is common in populations exposed to a range of parasites and wherever several parasite species occur at high frequency. These conditions apply most notably to socially and economically disadvantaged or marginalised communities, often rural, living in tropical and subtropical climate zones (Buck et al., 1978; Hotez et al., 2006; Petney and Andrews, 1998; Schratz et al., 2010). Multiparasitism has been acknowledged for over half a century already (Buck et al., 1978; Stoll, 1947). Indeed, the phenomenon of multiparasitism has been studied throughout the world (Petney and Andrews, 1998) with particular emphasis on Africa (Chunge et al., 1991, 1995; Keiser et al., 2002; Petney and Andrews, 1998; Raso et al., 2004; Tchuem Tchuenté et al., 2003; Thiong'o et al., 2001; Tshikuka et al., 1996; Utzinger et al., 1999). Multiparasitism has also been reported from the Americas (Ferreira et al., 1994; Guignard et al., 2000; Heukelbach et al., 2004; Jardim-Botelho et al., 2008) and eastern Asia (Auer, 1990; Sayasone et al., 2009b), including P.R. China (Booth et al., 1996; Yu et al., 1994). All studies consistently found that multiparasitism is the norm rather than the exception (Keusch and Migasena, 1982; King and Bertino, 2008; McKenzie, 2005; Singer and de Castro, 2007; Singer et al., 2007).

Notwithstanding its pervasiveness, relatively few studies have actually focused on the frequency and specific characteristics of multiparasitism. Indeed, it is routinely ignored in epidemiological surveys. Whilst it is common that prevalence data are presented on a per-species basis, multiparasitism rates and significant associations of parasite species as well as their clinical implications are rarely explored (Cox, 2001; Petney and Andrews, 1998). The large-scale surveys periodically undertaken in P.R. China may serve as an illustration. During the first nationwide survey pertaining to human intestinal parasites in P.R. China, carried out between 1988 and 1992, faecal samples of almost 1.5 million individuals were screened by the Kato-Katz technique, and Lugol-stained or saline direct smears were prepared. Among those infected with at least one parasite, 43.3% were found to harbour up to nine different species concurrently (Yu et al., 1994). The second national survey, carried out between 2001 and 2004, documented a significantly lower prevalence of common STHs, but unfortunately, no information is available on intestinal protozoa, and multiparasitism rates were not published in the peer-reviewed international literature (Ministry of Health, 2005).

Multiparasitism is also neglected in most attempts to estimate the local, regional or global burden of disease, which routinely focuses on individual parasites and ignores interactions between concurrently present species (Brooker and Utzinger, 2007; King and Bertino, 2008). Important underlying reasons are that interactions between parasite species and their influence on morbidity are ill-defined, and hence difficult to assess. Nevertheless, recent publications have highlighted the public health and wider socio-economic implications of multiple species infections (Drake and Bundy, 2001; Ezeamama et al., 2005a,b, 2008; Hotez et al., 2007; Jardim-Botelho et al., 2008; Molyneux, 2006; Mupfasoni et al., 2009; Pullan and Brooker, 2008). In the Philippines, for example, it was found that even light-intensity multiple helminth species infections resulted in a higher risk of anaemia and caused cognitive impairments among children (Ezeamama et al., 2005a,b, 2008). In recent years, the issue of multiparasitism has gained more pointed interest (McKenzie, 2005) despite the lasting limitations articulated by Keiser et al. (2002): (i) there is no single standardised diagnostic technique to simultaneously screen for all parasites or at least all intestinal parasites with high sensitivity; (ii) studies usually focus on schoolchildren or other subgroups of the population rendering generalisations difficult, and (iii) the attribution of commonly encountered signs of morbidity to particular parasites is often impossible.

2.2.2. Triggers for multiparasitism

A simultaneous infection with different parasites can result from chance events, that is, each infection is independent from other infections. High prevalences of several parasites in the same population (co-endemicity) must result in individuals infected by multiple species whenever the cumulative prevalence in the population exceeds 100% even if infections are not clustered. Significant co-infection rates, that is, the common occurrence of pairs or groups of parasites at higher frequency than independent probability events predict, result from a range of conditions (Howard et al., 2001; Petney and Andrews, 1998). Significant associations between different helminths, most notably, *A. lumbricoides* and *T. trichiura* (Booth and Bundy, 1992; Booth et al., 1998; Fleming et al., 2006; Howard et al., 2001; Needham et al., 1998; Raso et al., 2004; Tchuem Tchuenté et al., 2003) or intestinal protozoan species (Chunge et al., 1995; Utzinger et al., 1999) have been documented. They might be driven by common risk factors for infection with the implicated parasites (e.g. lack of clean water and improved sanitation, inadequate foot protection and not sleeping under a mosquito net) or ways of transmission common to several parasites (e.g. faeco-oral transmission, skin penetration and mosquito bites). Genetic and immunological predisposition to parasite infections may be another reason for high numbers of parasites and the presence of different species in certain individuals (so-called 'wormy people'; Cox, 2001; Ellis et al., 2007; Pullan et al., 2008; Stothard et al., 2009).

2.3. DIAGNOSTIC CHALLENGES IN THE STUDY OF MULTIPARASITISM

2.3.1. Multiparasitism—where and how it is studied

The most diverse parasite community is usually found in the digestive tract, but parasites can be found in virtually every niche offered by the human body, including the blood, lymph, cerebrospinal fluid, various tissues and organs and on the body surface.

The collection of urine, sputum and stool samples is less invasive than that of blood or other biological samples where eggs, larvae or adult parasites can be detected. Urine specimens are commonly used for the diagnosis of *Schistosoma haematobium* (the blood fluke causing urinary schistosomiasis; Gryseels et al., 2006), and sputum is employed for the diagnosis of *Paragonimus* spp. (lung flukes causing paragonimiasis) infections (Liu et al., 2008). Faecal samples are effectively used for the diagnosis of a wide range of intestinal parasites (Bergquist et al., 2009; Bethony et al., 2006; García, 2007). Many techniques for the diagnosis of parasites in stool are quite broad in scope, and hence facilitate the concurrent diagnosis of multiple species. Intestinal multiparasitism has therefore been studied more often and in greater detail than multiparasitism in any other niche, not to mention in its entirety. This has resulted in some inconsistency in the usage of terms in the scientific discourse, 'multiparasitism' sometimes being used when in fact 'intestinal multiparasitism' is referred to.

The assessment of the 'true' extent of multiparasitism in a population is challenging. Routine data from health care institutions (e.g. hospitals) are biased, since curative medicine focuses on sick individuals rather than random population samples, and a thorough diagnostic work-up is seldom possible in basic facilities. For study purposes, it may be feasible to screen a small number of individuals, usually a sample of a well-defined subgroup of a particular population, for the full range of parasites infecting humans. However, such a study will usually not be representative and the sample size too small due to logistical and financial constraints resulting from the need to repeatedly collect samples of different biological origin from the same study participant and screen them with a considerable number of diagnostic tools to elucidate the 'true' extent of multiparasitism.

The assessment of multiparasitism is also complicated by the similar morphology of many recognised parasite species or their eggs and cysts (e.g. *Taenia* spp. eggs, hookworm eggs, *Entamoeba histolytica* and *E. dispar* cysts). Even recognised entities like *Blastocystis hominis* might actually be a complex of several species (Tan, 2008), which can only be kept apart using molecular tools. Many other parasites have never undergone such scrutiny and may well reveal further biological complexity.

2.3.2. Diagnostic approaches to the study of multiparasitism

We currently lack simple, yet sensitive diagnostic tools even for common parasites, let alone a single technique capable of detecting *all* human parasites possibly present in a sample. Traditionally, parasitic infections have been diagnosed microscopically, either directly (e.g. ectoparasites and direct faecal smear) or after processing of the samples (e.g. stool sedimentation after ether concentration and FLOTAC) and clearing or staining (e.g. Kato-Katz thick smear of faecal samples, blood films and tissue samples). Visualisation using ultrasonography, X-ray, computed tomography (CT) or magnetic resonance imaging (MRI) has become important for the diagnosis of tissue- and organ-dwelling parasites and schistosomiasis.

A major limitation of many traditional parasitological techniques is their rather low sensitivity when employed on a single sample and whenever the infection intensity is low (Bergquist et al., 2009; Knopp et al., 2008). This issue can at least partially be attributed to the non-random temporal presence and spatial distribution of detectable parasitic elements (e.g. eggs, larvae and adult parasites; Booth et al., 2003; de Vlas and Gryseels, 1992; Marti and Koella, 1993; Nielsen and Mojon, 1987; van Gool et al., 2003; Yu et al., 1998) and the generally small size of samples screened in a diagnostic procedure. For example, on average, only 41.7 mg stool are used for a single Kato-Katz thick smear (Katz et al., 1972). Also, infestations by male nematodes or schistosomes only, as well as very recent infections, will routinely go undetected since no eggs are excreted. Other techniques, for example those based on molecular biological methods, are often more sensitive, but usually come at a higher cost and require trained manpower and infrastructure that are not always available in endemic settings. Tests relying on the indirect detection of parasites, namely those focusing on parasite antigens and specific antibodies, usually do not allow distinguishing between past and present infections, and there also is a time lag between infection and positive results upon testing. Moreover, these techniques often lack specificity (Doenhoff et al., 2004).

It has been argued that the choice of diagnostic approach must be dynamic, reflecting current circumstances, that is, expected parasites, their prevalence and infection intensity, and also take into account confounding factors like control interventions (Bergquist et al., 2009). While the selection of the diagnostic techniques to be employed thus depends on the biological sample, the suspected parasites, idiosyncrasies, as well as the available human, financial and technical resources, their overall sensitivity can often be boosted by screening multiple samples collected at different time points (Booth et al., 2003; de Vlas and Gryseels, 1992; Marti and Koella, 1993; Nielsen and Mojon, 1987; van Gool et al., 2003; Yu et al., 1998).

In view of these challenges, studies on multiparasitism usually limit their scope, focusing on one or two types of biological samples, collection of single or, if resources permit, multiple specimens from the same individual over a short time period, and screening of samples with several complementary diagnostic techniques (Steinmann et al., 2008). The more extensive the sampling and diagnostic efforts, the more complex and arguably prone to mistakes the study becomes, but the more accurate the extent of multiparasitism can be determined. Most studies rely on stool samples to investigate intestinal helminthiasis and schistosomiasis, sometimes complemented by screening for intestinal protozoa (Guignard et al., 2000; Keiser et al., 2002; Steinmann et al., 2008; Waikagul et al., 2002) and the assessment of urine (Bowie et al., 2004; Tchuem Tchuenté et al., 2003) or blood (Brooker et al., 2007; Raso et al., 2004; Tshikuka et al., 1996) samples for *S. haematobium* and malaria respectively.

To overcome this time- and resource-straining approach, sensitive, broad-spectrum diagnostic tools need to be developed to assess the 'true' extent and significance of multiparasitism. While a host of new parasite-specific molecular tools have been developed in recent years, little emphasis has been placed on parasitological methods suitable for the detection of multiple species concurrently. The development of the FLOTAC method for the diagnosis of helminth and intestinal protozoan infections in livestock (Cringoli, 2006), and its recent successful application for the diagnosis of human hookworm infections (Utzinger et al., 2008) and multiple intestinal helminth infections (Knopp et al., 2009a,b) holds promise to facilitate the evaluation of intestinal multiparasitism in humans.

2.4. MULTIPARASITISM IN EASTERN ASIA

2.4.1. General observations

The number and diversity of human parasites in eastern Asia is probably unsurpassed by any other region, not least because of a diversity of zoonotic helminths that proliferate due to permissive socio-cultural habits, including a widespread predilection for uncooked meat, fish and other dishes made of cultured, domesticated and wild animals (Liu and Boireau, 2002; Lv et al., 2008; Zhou et al., 2008). High population density, poverty, inadequate hygiene practises, lack of access to clean water and adequate sanitation infrastructure, favourable agricultural practises such as the use of human excreta as fertilizer ('night soil'), and local climatic conditions are additional supportive factors for high parasite prevalences. Historically, human parasitic infections and, arguably, multiparasitism were rampant throughout eastern Asia (Ohta and Waikagul, 2006). Today, the endemic areas and populations are more fragmented, with lower prevalences in socio-economically advanced societies compared to their more traditional peers. In P.R. China, for example, the eastern and southern coastal provinces have undergone more rapid economic development and lifestyle change than central and western areas (de Silva et al., 2003; Ministry of Health, 2005). Parasitic diseases as a mass phenomenon are now largely absent from the most affluent countries, for example, Japan (Kasai et al., 2007; Ohta and Waikagul, 2006) and most parts of South Korea (Shin et al., 2008) as well as Taiwan (Yeh et al., 2001).

However, virtually all countries which successfully controlled parasitic diseases in the past reported the persistence of small numbers of autochthonous infections and an increase in certain zoonotic parasites over recent years (Keiser and Utzinger, 2005; Lun et al., 2005; Lv et al., 2008; Ohta and Waikagul, 2006; Shin et al., 2008; Yeh et al., 2001; Zhou et al., 2008). The current level of STH infections in North Korea might be a proxy for the situation in South Korea before effective control programmes were implemented (Li et al., 2006), and the country is implicated in the re-emergence of certain parasitic diseases in its southern neighbour (Shin et al., 2008). Importantly, the decline in parasitic and especially STH infections in Japan has not been attributed to the economic development of the country alone, but particularly to an effective control programme developed and implemented by dedicated associations, the local governments and the private sector (Ohta and Waikagul, 2006). The successful control of STHs in South Korea was modelled on this example (Hong et al., 2006).

2.4.2. Studies of human multiparasitism in eastern Asia

Evidence for the pervasiveness of multiparasitism in eastern Asia is widely available in the international scientific literature, but few surveys have been explicitly designed to study this issue in greater depth. In many reports, multiparasitism is referred to only summarily or implicitly, and detailed results, such as the frequency of multiple parasite infections, the highest number of parasites found in individual participants and significant associations between different species as well as clinical manifestations, are often lacking. Most studies focused on a single parasite species and failed to leverage the efforts and expenses to generate additional information. For example, it is common in surveys pertaining to *Schistosoma japonicum* in P.R. China that only schistosome eggs are reported but not those of intestinal helminths, which would be readily available with minimal additional efforts and little extra expenses (Wang et al., 2009). Intestinal multiparasitism was investigated most often, sometimes complemented by screening for blood-dwelling parasites and parasite-specific serum antibodies. Studies on ectoparasites are rare, and not a single epidemiological survey that concurrently focussed on intestinal parasites, blood- or tissue-dwelling parasites and ectoparasites could be identified in the extant literature.

Some of the most comprehensive investigations of multiparasitism in eastern Asia were conducted in the 1970s in Indonesia (Cross et al., 1976; Joesoef and Dennis, 1980; Joseph et al., 1978; Stafford and Joesoef, 1976) and in the Philippines (Cross et al., 1977) and focused on intestinal helminths and protozoa, malaria, microfilariae and sometimes *Toxoplasma gondii*. Most studies, however, only considered intestinal parasites, mostly helminths (Auer, 1990; Baldo et al., 2004; Higgins et al., 1984;

Kim et al., 2003; Lee et al., 2000; Pegelow et al., 1997; Toma et al., 1999). Simultaneous treatment of multiple helminth infections was investigated in Indonesia at an early stage (Partono et al., 1974). Innovative studies conducted in the Philippines investigated the relationship between multiple helminth infections (STHs and *S. japonicum*) and anaemia (Ezeamama et al., 2005b, 2008). Recognising the importance of multiparasitism, its effects were considered in investigations on child growth and cognitive impairment due to individual helminth species (Ezeamama et al., 2005a; McGarvey et al., 1992).

Historical data on multiparasitism in Vietnam, Cambodia and Lao People's Democratic Republic (Lao PDR) are rare, probably due to political circumstances. From Vietnam, a generally high prevalence of STHs leading to frequent double or triple infections, but low prevalences of intestinal protozoa, trematodes and cestodes have been reported (Goodrich, 1967; Kim et al., 1970). More recent data corroborate these findings (Needham et al., 1998; Nguyen et al., 2006; Olsen et al., 2006; Uga et al., 2005). Studies conducted in Vietnam which also include blood screening are very rare (Verle et al., 2003). Parasitological surveys with a focus on multiple species conducted in Lao PDR consistently reported a high prevalence and diversity of trematode and STH infections as well as a remarkable frequency of cestodes, while intestinal protozoa were found to be less prevalent (Giboda et al., 1991; Rim et al., 2003; Sayasone et al., 2009a; Sithithaworn et al., 2006; Vannachone et al., 1998). One study focusing on individuals with abdominal or hepato-biliary symptoms in hospital and community-based surveys found up to seven different intestinal parasite species in some participants (Sayasone et al., 2009b). It is conceivable that most reports considerably underestimated multiparasitism due to food-borne trematodes since the eggs of many species are microscopically undistinguishable. Few studies made an attempt to concurrently collect stool and other biological samples (Giboda et al., 1991). Even less data are available from Cambodia where STHs appear to be common while intestinal protozoa are less prevalent and trematodes and cestodes are spatially restricted (Lee et al., 2002; Sinuon et al., 2003). The spectrum of endemic parasites in north-eastern Thailand resembles that of neighbouring Lao PDR, but control programmes and socio-economic development have a longer history (Herter et al., 2007; Kasuya et al., 1989; Waikagul et al., 2002, 2008). Multiple intestinal helminth infections were found to increase the likelihood of malaria episodes in western Thailand (Nacher et al., 2002). Lower levels of intestinal multiparasitism are now found in many parts of the country, probably due to the implementation of control programmes and the provision of health education, clean water and adequate sanitation as well as broad socioeconomic development (Kitvatanachai et al., 2008; Ngrenngarmlert et al., 2007; Pitisuttithum et al., 1990; Wongstitwilairoong et al., 2007). However, high parasite prevalences and multiparasitism rates are still found in many areas as shown in a study in a filariasis-endemic area of southern Thailand (Loymek et al., 2004), and especially among ethnic minority groups where malaria has sometimes been assessed along with intestinal parasites (Nithikathkul et al., 2003; Piangjai et al., 2003).

Few studies with an explicit focus on multiparasitism in P.R. China are available in the peer-reviewed English literature. The first national survey on the epidemiology of intestinal helminth infections across P.R. China found a prevalence of infection of 62.6% in 1988–1992, 43.3% of whom were infected by multiple species. A total of 56 intestinal parasite species were identified and the highest number of parasite species found in a single individual was nine (Yu et al., 1994). The second survey implemented between 2001 and 2004 found multiple infections among 24.1% of all infected individuals. Up to six species were found in the same individual (Coordinating Office of the National Survey on Important Human Parasitic Diseases, 2008). Other studies have confirmed that multiparasitism is still common (Booth et al., 1996; Steinmann et al., 2008; Tang and Luo, 2003). One study explicitly considered the effects of multiple parasite infections when investigating *S. japonicum*, child growth and nutritional status (McGarvey et al., 1993). In the 1960s, parasitic infections and multiparasitism were common among Chinese living in Hong Kong (Grant, 1969).

2.5. MULTIPARASITISM: TWO CASE STUDIES FROM YUNNAN PROVINCE, P.R. CHINA

2.5.1. Multiparasitism across a rural county

The epidemiology of parasitic infections in Eryuan county in northwest Yunnan province, P.R. China (Fig. 2.1), was studied in 2005 when stool and serum samples were collected from 3220 individuals, aged 5–88 years, living in 35 randomly selected villages representing all parts of the county (Steinmann et al., 2007b,c).

A single stool sample was collected from each individual and subjected to the Kato-Katz thick smear method for diagnosis of intestinal helminth and *S. japonicum* infections. No attempt was made to diagnose intestinal protozoa. However, enzyme-linked immunosorbent assays (ELISAs) were used to screen serum samples for antibodies against *S. japonicum*, cysticerci (*T. solium* larvae) and *Trichinella* spp. *A. lumbricoides* was the most common parasite with a prevalence of 15.4%, followed by *Taenia* spp. (3.5%), *T. trichiura* (1.7%) and hookworm (0.3%). *S. japonicum* eggs were found in 2.7% of the screened inhabitants of the 13 known endemic villages. Of the study cohort, 18.8% were infected with a single intestinal parasite species, while 1.6% harboured two species

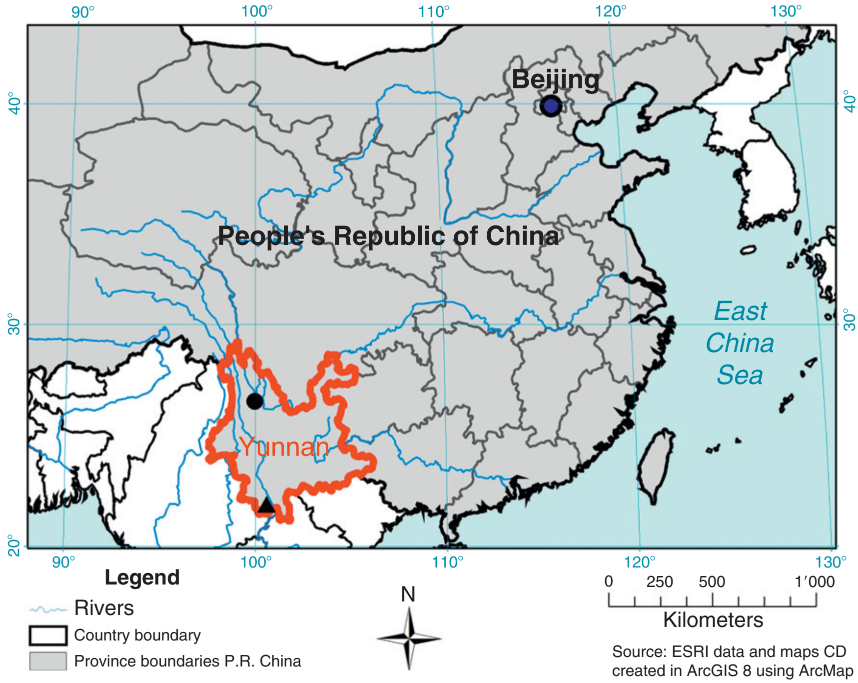

FIGURE 2.1 Location of Eryuan county (●) and Nongyang village (▲) in Yunnan province, P.R. China.

concurrently. Triple infections were found in 0.1% of the study participants (Table 2.1). The low frequencies of multiparasitism may be explained on the following grounds. Firstly, Eryuan county is located at altitudes ranging between 1950 and over 3000 m above sea level, hence winter temperatures are low, restricting the survival of free parasite stages, for example, hookworm. Secondly, only a single stool sample was collected and subjected to a single 41.7 mg Kato-Katz thick smear. It is widely acknowledged that multiple Kato-Katz thick smears and the combination of different stool examination techniques enhances the diagnostic sensitivity (Bergquist et al., 2009). Thirdly, Eryuan county has a history of large-scale administration of praziquantel for morbidity control due to schistosomiasis. The latter issue might explain why the seroprevalence of certain parasite infections was several-fold higher than what was found in stool examinations: 18.5% for cysticercosis and 49.5% for schistosomiasis japonica in known *S. japonicum*-endemic villages.

The study also revealed important spatial heterogeneities regarding the distribution of most investigated parasites. Explanatory factors were ethnicity, socio-economic status and village location with regard to elevation and slope. For example, most *A. lumbricoides* and *Taenia* spp.

TABLE 2.1 Results obtained from cross-sectional parasitological and serological surveys pertaining to helminth and intestinal protozoa infections in Eryuan county (Steinmann et al., 2007b) and Nongyang village (Steinmann et al., 2008), both located in Yunnan province, P.R. China

Characteristics	Prevalence	
	Eryuan county $n = 3220$ individuals	Nongyang village $n = 215$ individuals
Population profile		
Mean age (range) in years	34.7 (5–88)	29.0 (4–84)
Males	1427 (44.3%)	102 (47.4%)
Females	1793 (55.7%)	113 (52.6%)
Parasites		
Helminths		
Ascaris lumbricoides	15.4	92.6
Hookworm	0.3	88.8
Trichuris trichiura	1.7	88.8
Strongyloides stercoralis	Not determined	11.7
Enterobius vermicularis	0	7.4
Taenia spp.	3.5	5.1
Dicrocoelium dendriticum	0	1.4
Fasciolopsis buski	0	0.5
Schistosoma japonicum	1.3	Not endemic
Infection with ≥ 1 helminth species	20.5	100
Infection with 1 helminth species	18.8	4.2
Infection with 2 helminth species	1.6	15.4
Infection with 3 helminth species	0.1	62.3
Infection with 4 helminth species	0	16.7
Infection with 5 helminth species	0	1.4
Intestinal protozoa		
Blastocystis hominis	Not determined	20.0
Endolimax nana	Not determined	6.1
Entamoeba coli	Not determined	3.7
Iodamoeba bütschlii	Not determined	2.3
Giardia intestinalis	Not determined	1.9
Entamoeba hartmanni	Not determined	1.4
Entamoeba histolytica/E. dispar	Not determined	0.5
Infection with ≥ 1 protozoan species	Not applicable	24.2
Infection with 1 protozoan species	Not applicable	15.8

(*continued*)

TABLE 2.1 (*continued*)

Characteristics	Prevalence: Eryuan county $n = 3220$ individuals	Nongyang village $n = 215$ individuals
Infection with 2 protozoan species	Not applicable	5.1
Infection with 3 protozoan species	Not applicable	3.3
Seroprevalence		
Trichinella spp.	58.8	Not determined
Cysticercosis	18.5	Not determined
Schistosoma japonicum	27.1	Not determined
Evidence for parasite infection[a]	76.4	100
Parasite infection diagnosed	20.5	100

[a] Parasitological diagnosis or seropositivity.

infections, as well as most individuals with cysticerci-specific antibodies, were found in villages situated higher than 2150 m above sea level, that is, in distinctly poor and remote mountain villages often inaccessible by road and inhabited by the Bai ethnic minority. The sanitation infrastructure in these villages was either rudimentary or completely lacking. Indeed, faeco-oral transmission is the main risk factor for cysticercosis in humans and pigs, and *A. lumbricoides* is transmitted via contaminated soil or food. Those seropositive for *Trichinella* spp. showed opposite characteristics, that is, were relatively well-off and living in plain areas. This observation suggested that different animals or dishes were implicated in the transmission of cysticercosis and trichinellosis, respectively. *S. japonicum* was only found below 2150 m above sea level, mainly among the better-off Han nationality living in plain areas. However, *S. japonicum*-specific antibodies were commonly found beyond the recognised endemic area, indicating a vast potential for re-emergence of the disease once control programmes are discontinued.

This cross-sectional study showed the potential and limitations of a survey designed to assess multiparasitism across an entire county. On account of logistical and financial constraints, multiple stool samples could not be collected nor could the available sample be analysed with multiple diagnostic tools. Similarly, the range of immunological tests deployed for testing the serum samples against tissue- and blood-dwelling parasites had to be restricted, and ectoparasitism could not be investigated. Still, the survey provided a snapshot of the current status of intestinal and tissue-dwelling helminth parasites across Eryuan county.

Combined with the information collected by means of a questionnaire and remotely sensed environmental data, the available database clearly highlighted the significance of sanitation and transport infrastructure, or rather the lack thereof, and facilitated spatial and population-specific targeting of control programmes, health education and poverty alleviation schemes.

2.5.2. Intestinal multiparasitism in a single village

A case study of intestinal multiparasitism in a single village located in the southern part of Yunnan province is presented here in order to illustrate the efforts required to assess its 'true' extent even in a small community, and to highlight the shortcomings of current diagnostic tools (Steinmann et al., 2007a, 2008). The study was carried out in 2006 in Nongyang village, a Bulang settlement in Menghai county of the tropical Xishuangbanna prefecture (Fig. 2.1). The study aimed at collecting 2–3 stool specimens within 1 week from each of the 283 villagers aged 2 years and above. Each morning, stool specimens were collected and transferred to a nearby laboratory for detailed diagnostic work-up. The Kato-Katz thick smear technique (single slide; Katz et al., 1972), the Baermann apparatus (~20 g stool; García, 2007) and the Koga agar plate test (1–2 g stool; Koga et al., 1991) were employed for every stool sample. In addition, ~1 g of stool was conserved in sodium acetate–acetic acid–formalin (SAF; only one sample per participant; Marti and Escher, 1990) and was later examined for helminth eggs and intestinal protozoa using an ether-concentration method (García, 2007).

Upon the completion of the study, full records were available for 215 individuals in case the least-prioritised Baermann test was not considered, and 180 if it was. The difference can be attributed to the challenge of repeatedly collecting stool specimens of appropriate size for the Baermann test. Combining the results across all tests, a total of 15 different parasite species were detected; eight helminths (*A. lumbricoides*, *T. trichiura*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Taenia* spp., *Dicrocoelium dendriticum*, *Fasciolopsis buski*) and seven intestinal protozoa (*B. hominis*, *Endolimax nana*, *Entamoeba coli*, *Iodamoeba bütschlii*, *G. intestinalis*, *Entamoeba hartmanni*, *E. histolytica*/*E. dispar*). The prevalence of infection with individual parasites ranged from 0.5% (*E. histolytica/E. dispar*) to 92.6% (*A. lumbricoides*). The prevalences of both *T. trichiura* and hookworm infection exceeded 85%. The fourth-highest prevalence was found for *B. hominis*, reaching 20.0% (Table 2.1).

Among the 215 individuals, not a single person was free of parasite infections and only seven individuals (3.3%) were infected with a single species. Almost half of the study participants were simultaneously infected with three species (45.1%) and another 26.6% hosted four intestinal parasites concurrently. The maximum number of intestinal parasite species per individual was six; up to five helminths and as many as three

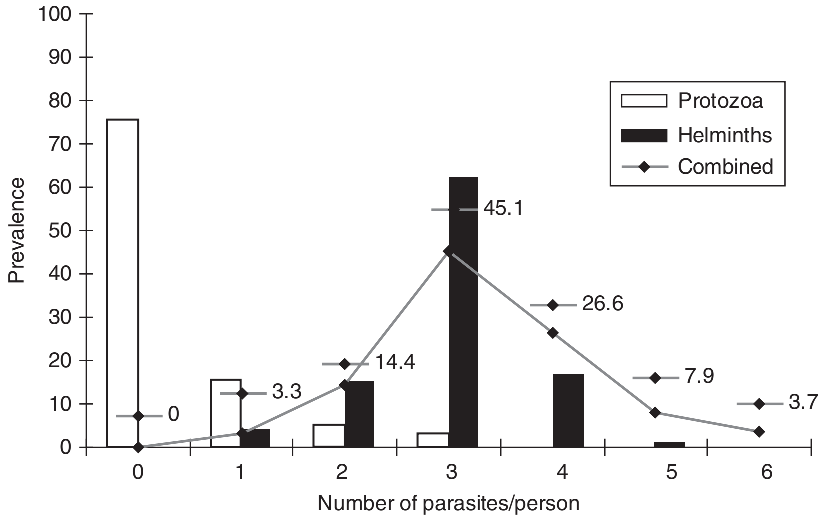

FIGURE 2.2 Prevalence of intestinal multiparasitism among 215 study participants from Nongyang village in Yunnan province, P.R. China.

intestinal protozoa (Fig. 2.2). No statistically significant differences were found between the number of helminths, intestinal protozoa or general parasites per person and sex or age group. However, a tendency for higher multiparasitism among elder participants was noted. Concurrent infections with three different intestinal protozoa, five helminth species or six intestinal parasites of any kind were only found in individuals aged 15 years and above. Table 2.1 contrasts the picture of multiparasitism as it was found in Nongyang village with that obtained through the previously discussed survey in Eryuan county. Of note, the obvious differences in parasite fauna, prevalences and multiparasitism rates are due to several independent factors, most importantly, the local environment, socioeconomic conditions, sampling strategy and employed diagnostic tools.

Different diagnostic tools were employed to detect the same parasites, thus the diagnostic performance of different methods could be compared. Additionally, 'true' infection prevalences could be estimated for several parasite species based on the distribution of positive diagnostic results among the stool samples collected from every individual who had submitted three stool specimens through the use of a simple mathematical model (Marti and Koella, 1993). The results suggested that most infections with STHs were identified with the approach taken, that is, the collection of multiple samples and their screening by different diagnostic methods. Collecting only one sample and analysing it with just one diagnostic tool would have seriously underestimated the prevalences of various parasites, most notably, *S. stercoralis* and hookworm.

Despite the rigorous diagnostic approach employed, the study still underestimated the full extent of multiparasitism in this community. The scarcity of epidemiological or clinical data on parasitic infections from this area, however, makes it impossible to estimate to which degree. The following points are offered for consideration. Firstly, the study focused on intestinal multiparasitism. No attempt had been made to investigate ectoparasites (e.g. lice, fleas and *S. scabiei*), and no blood samples had been collected; malaria is endemic in the southern part of Yunnan, though at a low prevalence. The presence of additional parasites primarily found in other biological samples (e.g. sputum, muscle and cerebrospinal system) must also be considered. Trichinellosis outbreaks have occurred repeatedly in this area, and the high prevalence of *Taenia* spp. infections, pig husbandry and low hygiene conditions are risk factors for cysticercosis. Secondly, only a single stool sample was screened for intestinal protozoa, employing an ether-concentration method. The concurrent analysis of the same samples for *B. hominis* by a more sensitive culture method resulted in an overall prevalence of 32.6% (Li et al., 2007), 1.6× higher than the prevalence according to the ether-concentration method (20.0%). We speculate that the prevalences of intestinal protozoa other than *B. hominis* were also underestimated. Thirdly, the sensitivity of the employed broad-spectrum diagnostic methods is evidently low for certain parasites, which require specialised approaches (e.g. *E. vermicularis*, *Taenia* spp.), while they altogether fail to detect additional groups of parasites (e.g. *Coccidia, Microspora*).

2.6. SIGNIFICANCE OF MULTIPARASITISM

2.6.1. Multiparasitism and health

Certain parasites cause overt acute or chronic morbidity and the resulting mortality can be high. Examples for such highly pathogenic parasites are lymphatic filariae, *P. falciparum*, *Trypanosoma* spp., *Leishmania* spp., *E. histolytica* and *Echinococcus* spp. Other parasites, however, cause little noticeable morbidity and infections seldom lead to death, most notably, many of the intestinal parasites (Bethony et al., 2006). For a long time, a view prevailed that many prevalent intestinal parasites do not cause morbidity, at least not in apparently healthy individuals and as long as infection intensities are low. This almost certainly is the case with several intestinal protozoa which are commensals rather than parasites (e.g. *Sarcodina*, except *E. histolytica*, *Mastigophora*, except *G. intestinalis* and possibly, *Dientamoeba fragilis*; Farthing, 2006). It is possibly also true for well-fed, otherwise healthy adults with low-intensity, single-species intestinal helminth infections. However, many parasites are most prevalent among the subgroups of society for which these conditions do not apply—children, women of child-bearing age, under- or malnourished people, those exposed to additional diseases or with a weakened immune system and those who are already parasitised by other species (Drake and Bundy, 2001; King and Bertino, 2008; Pullan and Brooker, 2008).

An important reason for the lasting neglect of the public health significance of intestinal parasitic infections is their non-specific character and chronic nature (King and Bertino, 2008). Iron-deficiency anaemia probably is the single most important negative clinical outcome of intestinal helminth (mainly hookworm) infections, adding to the already huge anaemia burden due to malaria (Hotez et al., 2006; Tolentino and Friedman, 2007). Malnutrition is another important effect of chronic intestinal helminthiasis and schistosomiasis, resulting in impaired growth and cognitive development, which negatively affect the socio-economic perspectives of whole societies who cannot realise their full potential (Hotez and Thompson, 2009; Hotez et al., 2009; Molyneux and Nantulya, 2004; Molyneux et al., 2005; Savioli et al., 2005). The morbidity resulting from each of several parasites dwelling in the same person may be additive, leading to negative health outcomes only if a certain threshold is passed (Mupfasoni et al., 2009). Recently, however, it has been demonstrated that even low-intensity infections with several intestinal helminths can cause significant morbidity among children (Ezeamama et al., 2005b, 2008). Other high-risk groups such as women of child-bearing age might be similarly affected. This suggests that health effects of certain parasites could be synergistic rather than additive (Fleming et al., 2006; Jardim-Botelho et al., 2008; Pullan and Brooker, 2008). It has also been observed repeatedly that multiparasitised individuals harbour higher numbers of worms of each species than those with single-species infections. Infection intensity, in turn, is closely linked to the severity of the resulting disease (Pullan and Brooker, 2008).

2.6.2. Multiparasitism and parasite control programmes

Today, the mainstay of helminth parasite control in endemic settings is preventive chemotherapy delivered through mass drug administration, targeting at-risk groups (e.g. school-aged children) or entire populations (Hotez et al., 2007; Utzinger and de Savigny, 2006). Current preventive chemotherapy programmes include the distribution of albendazole or mebendazole against STHs, praziquantel against schistosomes and ivermectin or diethylcarbamazine in combination with albendazole against lymphatic filariae (WHO, 2006). The integration of various control programmes to make them more (cost-)effective results in increasing co-administration of multiple drugs. For example, albendazole is now commonly distributed along with other drugs in the frame of lymphatic filariasis and schistosomiasis control programmes. Other drug combinations are also common (Brady et al., 2006; Molyneux et al., 2005; Utzinger and de Savigny, 2006). The concurrent distribution of several drugs has been shown to be generally safe, and hence no or only low levels of adverse events have been observed, even in areas with extensive multiparasitism (Lawrence, 2008; Mohammed et al., 2008; Olsen, 2007; Utzinger and Keiser, 2004). There are exceptions, however (García et al., 2003).

Certain challenges associated with multiparasitism and the indiscriminate distribution of drugs have not yet received wide attention and triggered limited research only. The following points are offered for discussion. Firstly, many parasites exhibit a focal distribution, that is, they tend to cluster geographically or among certain population strata. This necessitates the small-scale delineation of (co-)endemic areas and populations to avoid unnecessary costs and exposure to treatment with one or even multiple drugs (Brooker and Michael, 2000; Brooker and Utzinger, 2007; Brooker et al., 2002, 2006, 2007; Eziefula and Brown, 2008; Raso et al., 2006).

Secondly, the very success of preventive chemotherapy is likely to make this approach less cost-effective and justifiable over time since an ever-growing share of the target population will be free of (multiple-) infections and thus an increasing share of (multiple) drugs is administered unnecessarily (Raso et al., 2007). Therefore, regular evaluation of the current epidemiology of the target species should become an integral part of preventive chemotherapy programmes (Eziefula and Brown, 2008).

Thirdly, individual diagnosis is not performed in populations targeted by preventive chemotherapy campaigns, and such programmes often operate in areas and populations where the full spectrum of endemic parasites has never been assessed. While the employed drugs show good or at least satisfactory activity against the target parasites, this may not be the case with certain co-endemic species. Many anthelminthic compounds show limited activity against non-target parasites at commonly used treatment schedules (single dose) and dosage, for example, albendazole against *Taenia* spp. and *S. stercoralis* (Horton, 2000, 2002; Keiser and Utzinger, 2010). The efficacy of anthelminthic drugs against many non-target parasites has never been thoroughly investigated. It is thus likely that non-target parasite species are exposed to drugs in the course of preventive chemotherapy campaigns, sometimes at subcurative levels. This could facilitate the development of drug resistance, which is already a serious problem in veterinary medicine (Kaplan, 2004). No systematically impaired treatment efficacy of any anthelminthic drug has yet been observed in humans, but the situation needs to be closely monitored (Albonico et al., 2004; Geerts and Gryseels, 2000).

Fourthly, the treatment of entire populations against certain parasites may also lead to shifts in the spectrum and relative importance of endemic parasites. While the prevalences of susceptible parasites decrease in response to chemotherapy, less susceptible ones might overtake the freed niches or at least gain in relative significance (Kasuya et al., 1989). On the other hand, it is possible that non-target parasites are eliminated as well, resulting in ancillary benefits of the control interventions and an improved cost–benefit ratio. For example, new efforts for transmission control of *S. japonicum* in the lake and marshland areas of P.R. China which, among other measures, focus on clean water and improving sanitation, appear to have beneficial effects beyond the target disease schistosomiasis japonica: the local prevalence of STHs was reduced significantly (Wang et al., 2009).

2.7. MULTIPARASITISM: CURRENT RESEARCH NEEDS

Our current knowledge about multiparasitism and its implications for public health, control programmes and socio-economic development is limited (Pullan and Brooker, 2008). The most significant research needs as derived from the literature and our own research can be summarised as follows:

- There is a pressing need to deepen our understanding of the epidemiology and full extent of multiparasitism in different settings at the local, regional and global level. Geographical information system (GIS) and remote sensing offer new approaches for surveying and predicting parasite infections at different spatial scales. Significant parasite associations and the effects of multiple species infestations on the intensity of infection also need to be determined. Only the full appreciation of multiparasitism will allow determining its public health significance and impact on socio-economic development, equity and general well-being.
- Further progress in delimiting the extent of multiparasitism requires the development, validation and application of sensitive yet specific parasitological and molecular diagnostic tools, and their application in parasitological surveys, which need to be properly designed to assess the full spectrum of human parasites at different prevalence levels.
- New efforts in parasite systematics employing both conventional morphology and modern molecular tools are needed to re-assess traditional parasite entities and distinguish between morphologically similar species, which are possibly associated with distinct levels of morbidity.
- The true clinical, public health and socio-economic significance of multiple species parasitic infections needs to be evaluated using sensitive diagnostic tools, detailed knowledge of the epidemiology of multiparasitism and innovative approaches for determining and attributing impairments of growth, development, health and intellectual capacity. This knowledge is needed for an evidence-based appraisal of the true

global, regional and local burden of multiparasitism. To this end, acute and chronic, including subtle morbidity, impairments and psycho-intellectual effects resulting from single parasite infections need to be characterised, followed by the assessment of interactions between different parasite species and the health effects resulting thereof.
- The efficacy of currently available drugs for the treatment of parasite infections, especially those currently deployed in preventive chemotherapy campaigns, needs to be further studied with a focus on cure and egg reduction rates, as assessed with sensitive diagnostic tools. Their efficacies against parasites which are frequently associated with the target parasites or co-endemic in the population, also need to be thoroughly evaluated. Ancillary benefits of preventive chemotherapy consisting of the successful control of non-target parasites are currently under-researched. The efficacy and safety of drug combinations for the simultaneous treatment of co-endemic parasites as well as drug dosing have to be re-assessed in the light of broadly defined multiparasitism, and criteria for mass treatment revisited in the light of the results. Ultimately, the development of novel broad-spectrum antiparasitic agents is urgently needed, since the number of existing effective compounds for the treatment of most parasites is low, and back-up drugs are required to avert the loss of effective treatment options in case drug resistance develops and spreads.
- Drug resistance monitoring among a wide range of parasite species is required, since co-treatment of non-target parasites is likely wherever antiparasitic treatment is administered without prior diagnosis, multiparasitism is pervasive and drugs are employed that exhibit limited activity beyond their intended target. Clinical as well as molecular markers for drug resistance need to be identified, respective tests must be developed and awareness for emerging drug resistance increased among those responsible for surveillance.
- The endemic spectrum of parasites, including the relative importance of individual species and shifts therein following multiple rounds of preventive chemotherapy, must be monitored to readily adjust control programmes to the prevailing situation. Regular assessments of the local epidemiology and extent of parasitic infections have to accompany all preventive chemotherapy programmes, and long-term surveillance of multiparasitism is needed.

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