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Extensive Multiparasitism in a Village of Yunnan Province, People's Republic of China, Revealed by a Suite of Diagnostic Methods

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Abstract. Intestinal multiparasitism, the accuracy of different diagnostic techniques, and the influence of sampling effort were studied among 215 individuals in a Bulang village, Yunnan province, People's Republic of China. Behavioral, demographic, and socioeconomic data were obtained by questionnaire. Multiple stool specimens were examined by the Kato–Katz, Koga agar plate, Baermann, and ether–concentration methods. Eight helminth and 7 protozoa species were diagnosed. The prevalence of each of the 3 main soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) exceeded 85%. *Blastocystis hominis* was the most prevalent intestinal protozoan (20.0%). Over 80% of the individuals harbored 3 or more intestinal parasites concurrently. The infection intensities were predominantly light for hookworm and *T. trichiura* but moderate for *A. lumbricoides*. Examination of 3 instead of 1 stool specimen increased the sensitivity of helminth diagnosis, most notably for hookworm. Intestinal multiparasitism is rampant in this rural part of Yunnan province and calls for control measures.

INTRODUCTION

Multiparasitism is common among deprived populations, especially those living in developing countries.[1,2] Although this issue has been noted for decades,[3,4] contemporary epidemiologic investigations still focus on single-species infections or groups of related parasites.[5] Cross-sectional surveys usually list prevalence data for different parasites separately but neglect to report the extent of multiple-species parasite infections. Recently, the interest in the epidemiology of multiparasitism has grown, but the limitations put forward by Keiser and colleagues in 2002[6] still apply: (i) no single standardized diagnostic technique capable of detecting all intestinal, not to mention *all*, parasites with high sensitivity is available; (ii) most studies have focused on particular age groups; and (iii) little progress has been made in the attribution of specific symptoms out of the array of commonly encountered signs of morbidity to infections with particular parasites. Nevertheless, there is increasing awareness that new research is needed to deepen our understanding of multiparasitism, because even low-intensity infections with several helminths can cause significant morbidity.[2,7,8] Moreover, interactions between different parasites or common modes of transmission can result in notable associations between certain species.[6,9,10]

It is not feasible to screen entire populations for all potentially endemic parasites. However, concentrating on 1 or 2 kinds of biological samples and subjecting these to a suite of diagnostic approaches have proven useful. In most studies focusing on multiparasitism, stool samples were screened for helminth infections. Only a few studies examined stool samples for helminths and intestinal protozoa concurrently,[6,11] and only rarely was complementary blood testing done for the concurrent diagnosis of malaria.[9]

Although the approach taken depends on the parasites to be investigated, the sensitivity of common diagnostic techniques can be enhanced by screening multiple samples of suitable biofluids.[12–15] Thus, the combination of different diagnostic approaches and multiple samples holds promise to reveal the "true" extent of multiparasitism.[16]

The bulk of the published literature on intestinal multiparasitism stems from Africa.[6,9,17–19] Comparatively few data are available from Southeast Asia and People's Republic of China, at least on PubMed.[11,20–22] During the first national sampling survey on human parasites in China, carried out between 1988 and 1992, fecal samples of ~1.48 million individuals were screened by the Kato–Katz technique and Lugol-stained direct smears. Among those infected with at least 1 parasite, 43.3% were found to harbor 2–9 species concurrently.[23] The second national sampling survey, conducted between 2001 and 2004, documented a significantly lower prevalence of common soil-transmitted helminths, but, unfortunately, no information is available on intestinal protozoa.[24] Regarding soil-transmitted helminthiasis, data from the 2 surveys suggest that the focus had shifted from southeastern to central and western China. It is not known whether the epidemiology of intestinal protozoa followed the same trend.

The objective of this study was to assess the extent of intestinal multiparasitism in a village in southern Yunnan province, China. For this purpose, multiple stool specimens were examined with a suite of diagnostic approaches for the presence of helminths and intestinal protozoa. The design of our study allowed the investigation of the performance of different diagnostic techniques, and to study the influence of sampling effort on the recorded prevalence of single- and multiple-species parasitic infections.

MATERIALS AND METHODS

Study site and selection of participants. The study was carried out in May 2006 in Nongyang, a settlement belonging to the administrative village of Manguo in Menghai county, Xishuangbanna prefecture, Yunnan province, China (geographic coordinates: 100.35°E longitude, 21.81°N latitude). The village and the selection of the study participants have

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been described elsewhere.[25,26] In brief, Nongyang is located 1350 m above sea level. Smallholder animal husbandry and subsistence farming are common livelihood practices. In addition, people are engaged in tea and sugarcane farming for cash. The village possesses the basic infrastructure that is typically found in mountain settlements in this part of China: power, telephone, supply of untreated water at household level, and a community latrine. However, no family has its own latrine.

The local health authorities informed the village leaders about the aims and procedures of the study. After receipt of consent from local authorities and the village registry listing the 150-plus local households, all members of the 78 families with uneven registration numbers were enrolled in cohorts of 20–30 families per week.

Field and laboratory procedures. Details on field and laboratory procedures have been described.[25,26] In brief, we administered pretested individual and household-level questionnaires to obtain data on demography, occupation, behavior, living conditions, and agricultural and household asset ownership. In parallel, prelabeled stool-collection containers were handed out to every participant. Stool specimens were collected every morning, and new containers were distributed with the goal to obtain 3 specimens per individual.

The stool specimens were brought to the laboratory and processed within a maximum of 12 hours post-collection. First, the presence of *Taenia* spp. proglottids was recorded. Second, a single Kato–Katz thick smear was prepared using 41.7-mg punched plastic templates.[27] Helminth eggs were enumerated on a per-species basis within 1 hour under a light microscope. Third, about 10 g of stool was used to perform the Baermann test.[28] Fourth, 1–2 g of stool was placed on an agar plate for evaluation according to the Koga method.[29] The latter 2 tests were mainly used for diagnosis of *Strongyloides stercoralis*, but hookworm larvae can also be detected on the Koga agar plates.[30] Finally, for each individual, 1–2 g of stool was thoroughly mixed with 10 mL of sodium acetate–acetic acid–formalin (SAF) solution and shipped to a Swiss reference laboratory for subsequent semiquantitative examination for helminth eggs and intestinal protozoa, using a standardized SAF–ether–concentration method.[31]

Statistical analysis. Data were double-entered and cross-checked using EpiData version 3.0 (EpiData Association, Odense, Denmark). After a number of internal consistency checks had been performed, the data were transferred to STATA version 9.2 (Stata Corp., College Station, TX), and all statistical analyses were done with STATA.

The final cohort comprised those individuals who had ≥ 2 Kato–Katz thick smear readings plus ≥ 2 Koga agar plate test results plus the result from the SAF–ether–concentration method. In addition, the Baermann test was required for the evaluation of *S. stercoralis*.

Prevalence of individual parasites and multiparasitism were determined on the basis of combined results from the different diagnostic methods. Infection intensity for helminths was calculated using egg counts from the Kato–Katz thick smears only, with stratification into light, moderate, and heavy infections according to cutoff values put forward by the World Health Organization (WHO).[32] Associations between prevalence or infection intensity classes and sex or age were investigated using Pearson's χ^2 statistics.

The performance of the diagnostic techniques was assessed based on the assumption that the combined results of all tests accurately reflected the true infection status. Individual tests were compared with this diagnostic "gold" standard and among each other by exploring the differences between the respective proportions. Multiple logistic regression with stepwise manual backward elimination of variables at a level of $P = 0.15$ according to the likelihood-ratio test (LRT) was used to test the associations of individual parasites with the remaining parasites, adjusted for sex and age. A family-level random effect was introduced to account for clustering of infections within families. The "true" prevalence of *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura* was calculated based on the multiple Kato–Katz thick smear readings and according to a mathematical model described elsewhere.[14] This model has been used before to estimate the "true" prevalence of soil-transmitted helminth infections[33] and *S. stercoralis* in the village studied here.[26]

Ethical considerations and treatment. The study was approved by the institutional review boards of the Swiss Tropical Institute (Basel, Switzerland) and the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Shanghai, China). Authorities of local medical services informed village leaders about the study procedures. After their consent to perform the study was obtained, field workers visited the homes of the selected families where detailed information about the study, including potential risks and benefits, was provided. Arising questions were answered. Voluntary participation and the option to quit the study at any moment without further obligation or negative consequence were emphasized. Written confirmation that full information had been provided and individual participation was voluntary (informed consent) was obtained from the head of each participating household or a literate substitute designated by the head of the family.

At the completion of the study and in accordance with the local treatment policies, health personnel of the responsible parasitic diseases control station in Menghai offered free treatment with the compound mebendazole (mebendazole 100 mg/tablet + levamisole hydrochloride 25 mg/tablet, 2 tablets per day for 3 consecutive days) to all individuals of age ≥ 5 years in Nongyang village.

RESULTS

Compliance and demographics. Seventy-one families with 283 individuals were present at the time of the survey, all of whom agreed to participate. Figure 1 shows how many individuals complied with multiple stool specimen submission, allowing different diagnostic methods to be performed. Overall, 251 individuals (88.7%) submitted at least 1 sufficiently voluminous stool sample to carry out all but the Baermann test. Complete datasets with 2 or 3 stool specimens were obtained from 215 (76.0%; final cohort) and 177 (62.5%) participants, respectively. The full range of diagnostic tests, including the Baermann method, could be performed for 234 ($\geq$ 1 sample, 82.7%), 180 ($\geq$ 2 samples, 63.6%), and 128 individuals (3 samples, 45.2%), respectively.

Females represented 52.6% of the final cohort. The age of the participants ranged from 4 to 84 years with a mean age of 29 years and proportions of 11.2%, 10.7%, 25.6%, 28.8%, and 23.7% for the age groups 4–9, 10–14, 15–24, 25–39, and $\geq$ 40 years, respectively. There was no significant difference by sex

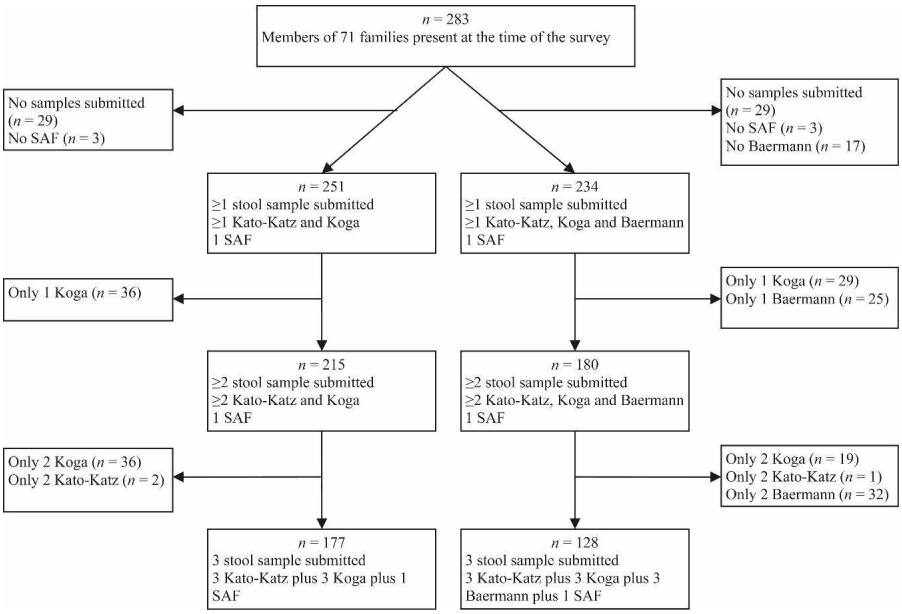

FIGURE 1. Compliance and number of diagnostic results among 283 inhabitants of Nongyang village in Yunnan province, China, who were invited to submit multiple stool specimens for screening by the Kato–Katz, Koga agar plate, Baermann and SAF–ether–concentration technique (only 1 sample for the latter).

among the age groups [$\chi^2 = 6.39$, degree of freedom (df) = 4, $P = 0.172$], and the age and sex distributions of the final cohort were similar to that of all 283 study participants (both $P > 0.05$).

Parasitic infections and multiparasitism. Screening of at least 2 stool specimens per individual using 4 different diagnostic tests resulted in detection of 15 intestinal parasite species: 8 helminths and 7 protozoa (Table 1). Very high prevalences were recorded for *A. lumbricoides* (92.6%), hookworm (88.8%), and *T. trichiura* (88.8%). The highest prevalences of *A. lumbricoides*, hookworm, and *T. trichiura* infections were found among individuals of age between 10 and 24 years, but only *T. trichiura* prevalence differed significantly among age groups ($\chi^2 = 19.86$, df = 4, $P = 0.001$). The prevalence of *S. stercoralis* was 11.7%; detailed results pertaining to this parasite have been presented elsewhere.[26] The remaining helminths identified were *Enterobius vermicularis* (7.4%), *Taenia* spp. (5.1%), *Dicrocoelium dendriticum* (1.4%), and *Fasciolopsis buski* (0.5%). *Taenia* spp. infections were found only in males ($P < 0.001$), among ≥ 15-year-olds, and steadily, albeit not significantly, increased with age ($\chi^2 = 7.11$, df = 4, $P = 0.119$).

The most common intestinal protozoan parasite was *Blastocystis hominis* (20.0%), followed by *Endolimax nana* (6.1%), *Entamoeba coli* (3.7%), *Iodamoeba bütschlii* (2.3%), *Giardia intestinalis* (1.9%), *Entamoeba hartmanni* (1.4%), and *Entamoeba histolytica*/*Entamoeba dispar* (0.5%). *G. intestinalis* was found only in individuals under age 25 years ($P = 0.109$), whereas *E. nana* was not found among participants younger than 15 years ($\chi^2 = 9.68$, $P = 0.046$).

Multiparasitism was very common; not a single individual was un-infected, and we identified up to 6 parasite species per individual: 1–5 helminths and as many as 3 intestinal protozoa. Almost half of the study participants harbored 3 parasite species concurrently (45.1%), and another 26.6% were infected with 4 species. Single-species helminth infections were rare (4.2%), 3 helminth species were diagnosed in 62.3% of the participants, and 5 species in 1.4%. On the other hand, no intestinal protozoa were diagnosed among three-quarters of the population sample, whereas triple-species intestinal protozoan infections were diagnosed in 3.3% (Figure 2). No significant differences were found between the number of helminth, protozoa, or general parasite species per person and sex or age, but there was a tendency for multiple-parasite species infections among elder participants; concurrent infections by 3 different protozoa, 5 helminth species, or 6 intestinal parasites of any kind were found only in participants ≥ 15 years of age (data not shown).

Helminth infection intensities. Because the Kato–Katz method is quantitative, infection intensities could be calculated for the major soil-transmitted helminths. The geometric mean number of eggs per gram stool (epg) among the infected was 7525 epg [95% confidence interval (CI) = 5850–9680 epg] for *A. lumbricoides*, 137 epg (95% CI = 114–164 epg) for hookworm, and 121 epg (95% CI = 103–144 epg) for *T. trichiura*. The infection intensities, stratified by sex and age group, are shown in Figures 3 and 4. The majority of *A. lumbricoides* infections were of moderate intensity (58.5%). Light and heavy infections made up 31.3% and 10.2% of all cases, respectively. Hookworm and *T. trichiura* infections were primarily of light intensity; 98.8% and 97.8%, respectively. There was no significant difference in the infection intensity of any parasite between males and females (all $P > 0.05$), but moderate-intensity hookworm infections were limited to males ≥ 25 years old. *A. lumbricoides* and *T. trichiura* infection intensities varied significantly between age groups ($\chi^2 = 22.88$, df = 12, $P = 0.029$; and $\chi^2 = 30.30$, df = 8, $P < 0.001$, respectively), whereas the hookworm infection intensity showed borderline significance ($\chi^2 = 15.32$, df = 8, $P = 0.053$). Heavy *A. lumbricoides* infections were mainly found among children of age 4–9 years (30% of this subpopulation versus 7.2% in all other age groups).

TABLE 1

Total prevalence of helminths and intestinal protozoa among 215 (*S. stercoralis*: 180) study participants from Nongyang village in Yunnan province, China, after screening ≥ 2 stool samples by the Kato–Katz, Baermann, Koga agar plate, and a SAF–ether–concentration method (only 1 sample/person), stratified by sex and age group*

Parasite	Prevalence in % (*n*)	95% CI	Pathogenic	Sex				Age (years)						
				Female	Male	χ^2	*P*†	4–9	10–14	15–24	25–39	≥ 40	χ^2	*P*†
Helminths														
Ascaris lumbricoides	92.6 (199)	89.0–96.1	Yes	95.6	89.2	3.15	0.076	95.0	95.2	96.4	91.9	87.5	3.72	0.445
Trichuris trichiura	88.8 (191)	84.6–93.1	Yes	91.2	86.3	1.29	0.257	90.0	100	92.7	95.2	73.2	19.86	0.001
Hookworm	88.8 (191)	84.6–93.1	Yes	88.5	89.2	0.03	0.867	75.0	95.2	96.4	88.7	83.9	9.35	0.053
Strongyloides stercoralis	11.7 (21)	6.9–16.4	Yes	6.1	18.3	6.42	0.011	0	0	19.6	9.4	15.9	8.70	0.069
Enterobius vermicularis	7.4 (16)	3.9–11.0	Yes	8.9	5.9	0.69	0.408	5.0	0	8.9	8.1	8.9	2.26	0.689
Taenia spp.	5.1 (11)	2.1–8.1	Yes	0	10.8	12.84	< 0.001	0	0	1.8	6.5	10.7	7.11	0.119
Dicrocoelium dendriticum	1.4 (3)	0–3.0	Yes	0.9	2.0	NA	0.605‡	0	0	0	3.2	1.8	NA	0.881‡
Fasciolopsis buski	0.5 (1)	0–1.4	Yes	0	1.0	NA	0.474‡	0	0	0	0	1.8	NA	0.712‡
Intestinal protozoa														
Blastocystis hominis	20.0 (43)	14.6–25.4	Uncertain	23.0	16.7	1.35	0.246	20.0	19.1	21.4	24.2	14.3	1.91	0.753
Endolimax nana	6.1 (13)	2.8–9.3	No	8.9	2.9	3.29	0.070	0	0	7.1	12.9	1.8	9.68	0.046
Entamoeba coli	3.7 (8)	1.2–6.3	No	5.3	2.0	1.68	0.195	10.0	0	5.4	3.2	1.8	4.06	0.398
Iodamoeba bütschlii	2.3 (5)	0–4.4	No	2.7	2.0	0.11	0.736	0	0	5.4	1.6	1.8	3.45	0.485
Giardia intestinalis	1.9 (4)	0–3.7	Yes	1.8	2.0	NA	1.000‡	5.0	4.8	3.6	0	0	NA	0.109‡
Entamoeba hartmanni	1.4 (3)	0–3.0	No	1.8	1.0	NA	1.000‡	0	0	3.6	1.6	0	NA	0.751‡
Entamoeba histolytica/E. dispar	0.5 (1)	0–1.4	Yes/No	0	1.0	NA	0.474‡	5.0	0	0	0	0	NA	0.093‡

* CI = confidence interval; NA = not applicable.
† Pearson's χ^2 test.
‡ Fisher's exact test.

Parasite associations. Table 2 shows the association of each parasite with the remaining ones, adjusted for sex and age. Study participants harboring *A. lumbricoides* were often co-infected with *T. trichiura* [odds ratio (OR) = 5.65, $P = 0.007$]. *B. hominis* was significantly associated with *E. vermicularis* (OR = 3.78, $P = 0.036$) and the protozoa *E. nana* and *E. coli.* It was also often found in individuals with *T. trichiura* (OR = 7.18), but this association did not reach conventional statistical significance ($P = 0.066$). Some nonpathogenic intestinal protozoa were less often found in people with particular helminth infections. *E. nana*, for example, was less prevalent among people with *A. lumbricoides* infections (OR = 0.17, $P = 0.045$), and hookworms were less often found among participants with an *I. bütschlii* infection (OR = 0.08, $P = 0.100$). However, the latter association did not reach standard statistical significance.

Diagnostic performance of different techniques. Table 3 shows the performance of individual diagnostic techniques, including comparison with our designated "gold" standard obtained from the pooled results of at least 2 methods for *A. lumbricoides*, hookworm, *T. trichiura*, and *S. stercoralis*, with findings for the latter parasite presented elsewhere.[26] The analysis of 2–3 stool samples with the Kato–Katz technique was slightly, but not significantly, more sensitive for *A. lumbricoides* (98.0%, 95% CI = 94.9–99.5%), *T. trichiura* (93.7%, 95% CI = 89.3–96.7%), and hookworm infection (84.8%, 95% CI = 78.9–89.6%) than the analysis of a single SAF-conserved sample by the ether–concentration method (*A. lumbricoides*: 93.0%, 95% CI = 88.5–96.1%; *T. trichiura*: 89.5%, 95% CI = 84.3–93.5%; hookworms: 77.5%, 95% CI = 70.9–83.2%). For the diagnosis of hookworm infection, the most sensitive method was the Koga agar plate technique. It detected 90.1% (95% CI = 84.9–93.9%) of all infections, significantly more than the SAF–ether–concentration method (77.5%, 95% CI = 70.9–83.2%; $P = 0.008$). The use of just 1 diagnostic method significantly underestimated the "true" prevalence for all parasite-diagnostic technique combinations, except for the diagnosis of *A. lumbricoides* and *T. trichiura* by the Kato–Katz technique.

The analysis of 3 instead of a single stool specimen considerably increased the sensitivity of the diagnostic tools (Table 4). The difference was most pronounced for diagnosis of hookworm infections. The measured prevalence increased from 43.6% (first sample) to 76.6% (third sample) and from 54.2% to 81.0% for the Kato–Katz and Koga agar plate methods, respectively. The corresponding sensitivity of a single test was 56.9% and 66.9%, respectively. Table 4 also lists the frequency of positive test results among the 3 samples collected from every individual and the derived "true" prevalences as well as sensitivities of the analysis of single or triple samples. The model predicted only marginally higher "true" prevalences than the actually recorded ones and, consequently, high sensitivities for the analysis of 3 samples. However, the estimated sensitivity of analyzing a single sample was considerably above the measured value for the diagnosis of hookworm infection by the Kato–Katz technique (measured, 56.9%; predicted, 70.3%).

DISCUSSION

We collected up to 3 stool specimens from 215 individuals living in a village in the southern part of Yunnan province,

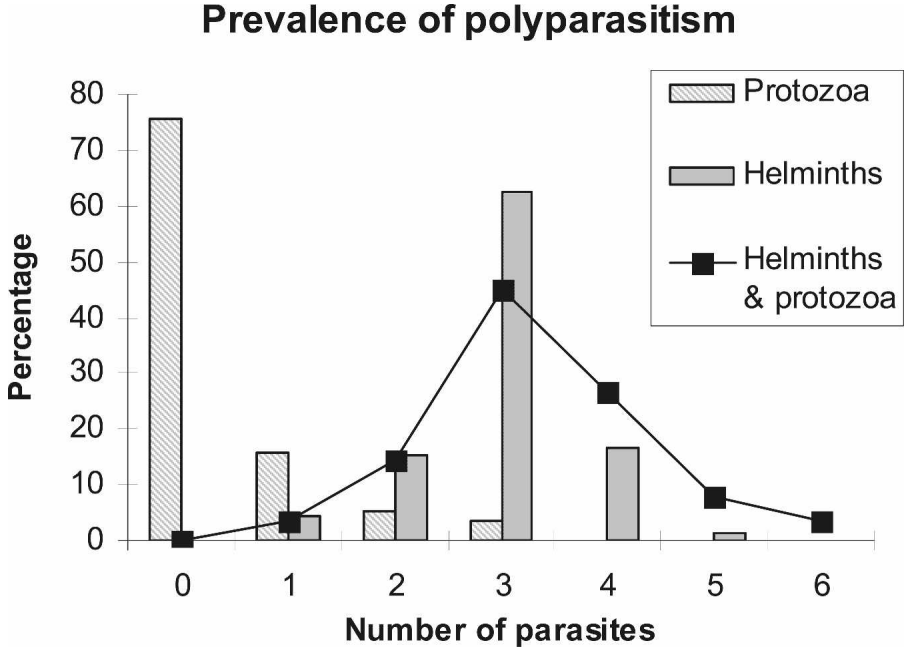

FIGURE 2. Prevalence of multiple species parasite infections among 215 study participants from Nongyang village in Yunnan province, China.

China. The stool samples were screened for helminths and intestinal protozoa using 4 different diagnostic methods, i.e., Kato–Katz, Baermann, Koga agar plate, and ether–concentration after conservation of the sample in SAF solution for 3 months. We found prevalences in excess of 85% for the 3 main soil-transmitted helminths. Five additional helminth species were recorded at prevalences ranging from 0.5% (*F. buski*) to 11.7% (*S. stercoralis*). Interestingly, much lower prevalences were recorded for intestinal protozoa than for the 3 main soil-transmitted helminths, the most common one being *B. hominis*, which parasitized one-fifth of the study cohort. Consequently, multiple-species helminth infections were the norm, but multiple intestinal protozoa infections were the exception. Our study population harbored up to 6 different parasites concurrently, and among those who submitted at least 2 stool samples we found none who was free of an infection. The infection intensities were predominantly light for hookworm and *T. trichiura* but moderate for *A. lumbricoides*. For the latter parasite, we also noted heavy infections. The maximum mean epg value was 191,700, counted in the stool samples of a 28-year-old female.

The low prevalence of pathogenic intestinal protozoa in this part of the world has been noted before[20] but has not attracted wide attention, and we are not aware of any explanation for this observation. Giboda and colleagues[20] also pointed out a high prevalence of multiple species helminth infections. Country-wide surveys among schoolchildren in neighboring Myanmar and Lao PDR employing the Kato–Katz technique on a single stool sample reported very high prevalences of soil-transmitted helminths. Interestingly, the survey in Myanmar[34] noted the lowest prevalences in the hilly area, which includes ecological zones that are similar to our

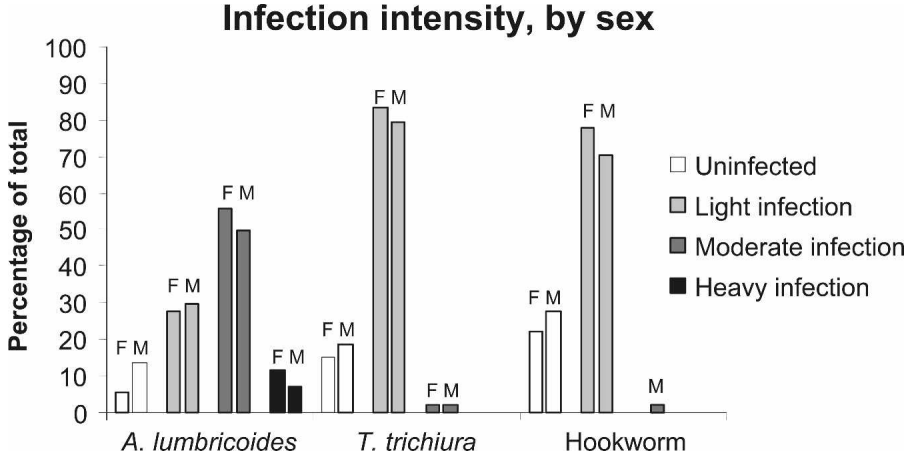

FIGURE 3. Infection intensity of *A. lumbricoides*, *T. trichiura*, and hookworm, stratified by sex, among 215 study participants from Nongyang village in Yunnan province, China. Mean of 2–3 stool samples assessed by the Kato–Katz method and classified according to WHO guidelines[32] (F, females; M, males).

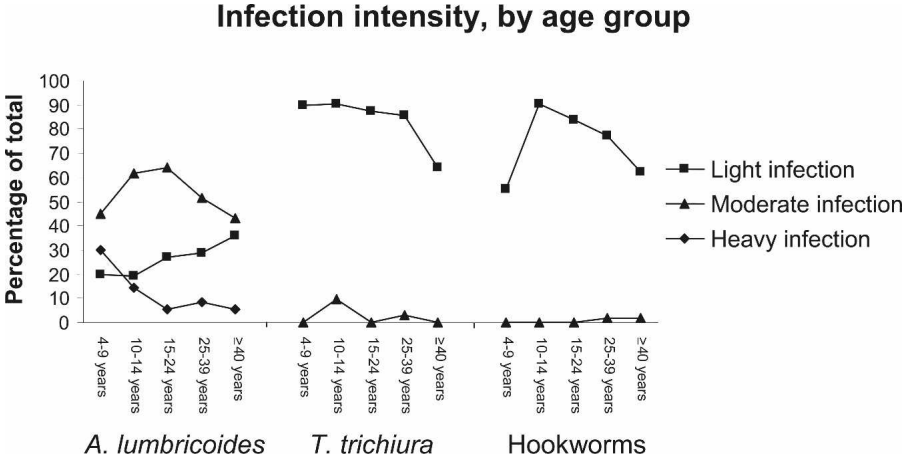

FIGURE 4. Age group-stratified infection intensity of *A. lumbricoides*, *T. trichiura*, and hookworm among 215 study participants from Nongyang village in Yunnan province, China. Mean of 2–3 stool samples assessed by the Kato–Katz method and classified according to WHO guidelines.[32]

study area. In Lao PDR, the highest prevalences of soil-transmitted helminths were reported from the northern provinces,[35] which share many eco-epidemiologic characteristics with Xishuangbanna prefecture in China. It should be noted that the environmental and epidemiologic conditions of southern Yunnan are distinct from other parts of the province. Hence, generalizations are difficult. This can be illustrated by comparing the results from the current study to data obtained during a parasitologic survey in Eryuan county in north-western Yunnan. The Eryuan study included 35 villages situated at elevations between 1750 m and 2700 m, and we found an overall prevalence of helminth infections of 20.5%.[36]

There are several limitations to our study. Together with issues already covered before,[26] we offer the following points. An important gap is the infection status of children under the age of 4 years. Families were reluctant to enroll their young children for the study, and it is difficult to obtain large-enough stool samples from this age group. Therefore, we are not in a position to conjecture whether multiparasitism is an issue among infants and preschoolers, and hence the age of infection for intestinal parasites remains to be investigated. Further, it could be argued that we overestimated the "true" infection intensities but underestimated prevalences because of false-negative results from people with low-intensity infections for which the Kato–Katz technique lacks sensitivity.

TABLE 2

Stepwise multiple logistic regression using backward elimination at a level of $P = 0.15$ to investigate associations between individual parasites and the remaining parasites, sex, and age group among 215 study participants from Nongyang village in Yunnan province, China

Parasite	Associations ($P < 0.15$)	OR (95% CI)	P
Helminths			
Ascaris lumbricoides	*Trichuris trichiura*	5.65 (1.61–19.80)	0.007
	Endolimax nana	0.17 (0.03–0.96)	0.045
	Iodamoeba bütschlii	0.15 (0.02–1.21)	0.075
	Sex: Female	1.00	
	Male	0.29 (0.08–1.00)	0.050
Trichuris trichiura	*Ascaris lumbricoides*	4.34 (1.14–16.50)	0.031
	Blastocystis hominis	7.18 (0.88–58.94)	0.066
	Age	0.55 (0.35–0.88)	0.012
Hookworm	–	–	
Strongyloides stercoralis	Sex: Female	1.00	
	Male	3.04 (1.20–7.70)	0.019
Enterobius vermicularis	*Blastocystis hominis*	2.65 (0.87–8.02)	0.085
Taenia spp.	–	–	
Intestinal protozoa			
Blastocystis hominis	*Enterobius vermicularis*	3.78 (1.09–13.11)	0.036
	Endolimax nana	15.57 (3.02–80.36)	0.001
	Entamoeba coli	14.66 (2.15–100.09)	0.006
	Iodamoeba bütschlii	11.98 (0.77–187.45)	0.077
Endolimax nana	*Blastocystis hominis*	13.40 (3.34–53.79)	< 0.001
	Iodamoeba bütschlii	11.22 (1.31–96.28)	0.028
Entamoeba coli	*Blastocystis hominis*	13.86 (2.68–71.68)	0.002
Iodamoeba bütschlii	Hookworm	0.08 (< 0.0–1.62)	0.100
	Blastocystis hominis	10.37 (0.70–154.54)	0.090
	Endolimax nana	18.28 (1.50–223.44)	0.023

CI = confidence interval; OR = odds ratio.

TABLE 3

Performance of individual techniques for the diagnosis of soil-transmitted helminth infections compared with our designated diagnostic "gold" standard among 215 individuals from Nongyang village in Yunnan province, China

Parasite	*A. lumbricoides*			*T. trichiura*			Hookworm			
Test	Kato–Katz (2–3 samples)	SAF–ether–concentration (1 sample)	"Gold" standard ("true" prevalence)*	Kato–Katz (2–3 samples)	SAF–ether–concentration (1 sample)	"Gold" standard ("true" prevalence)*	Kato–Katz (2–3 samples)	SAF–ether–concentration (1 sample)	Koga agar plate (2–3 samples)	"Gold" standard ("true" prevalence)*
Prevalence (%)	90.7	86.0	92.6	83.3	79.5	88.8	75.3	68.8	80.0	88.8
95% CI†	(86.8–94.6)	(81.4–90.7)	(89.1–96.1)	(78.3–88.2)	(74.1–84.9)	(84.6–93.0)	(69.6–81.1)	(62.6–75.0)	(74.7–85.3)	(84.6–93.0)
Sensitivity (%)	98.0	93.0	100	93.7	89.5	100	84.8	77.5	90.1	100
95% CI	(94.9–99.5)	(88.5–96.1)		(89.3–96.7)	(84.3–93.5)		(78.9–89.6)	(70.9–83.2)	(84.9–93.9)	
Difference‡	1.9	6.5		5.6	9.3		13.4	20.0	8.8	
95% CI	(–3.4 to 7.1)	(0.7–12.3)		(–0.9 to 12.1)	(2.5–16.1)		(6.4–20.6)	(12.5–27.5)	(2.0–15.6)	
P	0.486	0.029		0.095	0.008		< 0.001	< 0.001	0.012	
Negative predictive value (%)	80.0	53.3		66.7	54.6		45.3	35.8	55.8	
95% CI	(56.3–94.3)	(34.3–71.7)		(49.0–81.4)	(38.9–69.6)		(31.6–59.6)	(24.5–48.5)	(39.9–70.9)	

* "Gold" standards: for *A. lumbricoides* and *T. trichiura*, combined results of Kato–Katz plus SAF–ether–concentration; for hookworm, combined results of Kato–Katz plus SAF–ether–concentration plus Koga agar plate.
† CI = confidence interval.
‡ Difference between measured prevalence and true prevalence.

However, we are confident that this was not a major issue because the measured prevalence differed only slightly from the estimated "true" values, and the latter were somewhat lower than the combined results from all techniques. It is also conceivable that we slightly overestimated the sensitivity of the evaluated diagnostic methods because no absolute diagnostic "gold" standard was available. Instead, we considered the combined results from the different approaches as our reference. The very high prevalence of all considered parasites does not leave room for large differences between our assumed and the "true gold" standard and therefore renders it unlikely that overestimation of the performance of single techniques was a major issue. The prevalence of *E. vermicularis* was certainly underestimated because we did not use the standard diagnostic approach for this helminth, i.e., the Scotch-tape method.[28] Finally, the absolute strengths of the identified associations between parasites should not be overvalued because they result from multiple analyses.

It is widely acknowledged that the analysis of a single stool sample by only one technique results in a considerable number of false-negative results.[15,16,37–39] The benefits associated with analyzing multiple stool samples by different diagnostic approaches have been confirmed in our study for helminth infections. For intestinal protozoa, however, we tested a single stool sample by only 1 technique. Therefore, we anticipate that the prevalence of intestinal protozoa has been underestimated. This can be illustrated for *B. hominis*, for which we recorded a prevalence of 20.0%, whereas the screening of the same samples by a more sensitive culture method resulted in a prevalence of 32.6%.[25] Similar or even larger discrepancies between the measured and the "true" prevalence must be assumed for other intestinal protozoa. Our results indicate that under the prevailing conditions, the screening of 3 stool samples by 2–3 methods is likely to detect most helminth infections. Because we used only the SAF–ether–concentration technique for the diagnosis of intestinal protozoa, we are unable to comment whether it is sufficient to analyze 3 stool samples by this technique or if multiple techniques should be used.

The reliable diagnosis of hookworm infections is more difficult compared with *A. lumbricoides* and *T. trichiura*, particularly if only 1 stool sample is available or a several-hour time delay occurred between stool production, sample collection, and analysis.[40,41] The results of 3 methods (Kato–Katz, SAF–ether–concentration, and Koga agar plate) applied to 2–3 stool samples were available to us, and we observed individual sensitivities of 77.5–90.1% when compared with the overall result. Interestingly, culture on agar plates exhibited the highest sensitivity, a result that could not be expected based on the limited prior experience with this method.[30] It should further be noted that we tested 3 stool samples by the Koga agar plate and the Kato–Katz techniques, but only 1 SAF-conserved sample by ether–concentration. Our result strongly suggests that screening for *S. stercoralis* by the Koga agar plate method has additional benefits for detection of hookworm infections. Employing this technique would also allow differentiation of hookworm species, i.e., *Ancylostoma duodenale* and *Necator americanus*, if desired.

Sensitive diagnostic methods that reliably detect the presence of infections of any intensity are not only important for individual diagnosis but should also be consistently used in epidemiologic studies and surveys at population level. They

TABLE 4

Influence of the collection and analysis of multiple stool samples on the observed prevalence, estimated "true" prevalence and sensitivity of the diagnostic technique; samples were collected among inhabitants of Nongyang village in Yunnan province, China

	Kato–Katz									Koga agar plate		
	A. lumbricoides			*T. trichiura*			Hookworm			Hookworm		
Feature	No.	%	Sens.*	No.	%	Sens.*	No.	%	Sens.*	No.	%	Sens.*
No. of samples analyzed	188	100		188	100		188	100		179	100	
Cumulative prevalence after analysis of												
1st stool sample	156	83.0	91.8	114	60.6	71.3	82	43.6	56.9	97	54.2	66.9
2nd stool sample	167	88.8	98.2	147	78.2	91.9	123	65.4	85.4	132	73.7	91.0
3rd stool sample	170	90.4	100	160	85.1	100	144	76.6	100	145	81.0	100
Helminth eggs/larvae detected in												
1 stool sample	10	5.9		27	16.9		36	25.0		40	27.6	
2 stool samples	11	6.5		51	31.9		48	33.3		51	35.2	
3 stool samples of positive participants	149	87.6		82	51.2		60	41.7		54	37.2	
Estimated prevalence (%)†		90.5 ± 4.3			86.1 ± 5.3			78.7 ± 6.4			83.9 ± 6.2	
Sensitivity (single sample, %)		93.9 ± 2.1			77.2 ± 4.1			70.3 ± 4.8			67.5 ± 5.0	
Sensitivity (3 samples, %)‡		100			98.8			97.4			96.6	

* Sens. = sensitivity; "gold" standard: 3 samples analyzed with the respective method.
† Prevalence and performance indicators of diagnostic tests estimated using a mathematical model described by Marti and Koella (see Ref. 14).
‡ Measured versus predicted prevalence.

are particularly valuable for baseline studies and the evaluation of treatment interventions. At baseline, it is important to know the "true" prevalence of all parasites in the population, including their distribution among population subgroups, to tailor control programs to the local conditions, thereby enhancing the cost-effectiveness of the intervention. At follow-up, the use of sensitive diagnostic tools is paramount for the detection of the predominantly low-intensity infections among individuals who had not been cured completely.[42,43] We speculate that, currently, the prevalence at baseline is often underestimated whereas the efficacy of drug treatment is overestimated because the relative proportion of difficult-to-detect low-intensity infections is likely to be higher after chemotherapeutic interventions.[32]

Periodic treatment of millions of people with anthelminthic compounds[44–46] and improved socioeconomic conditions[47] have reduced the mean infection intensity in many parts of the world. Collection of multiple stool samples and analysis by different methods is one option to improve the sensitivity of common diagnostic tools. Another approach would be development of novel diagnostic techniques. Little emphasis has been placed on the development of new diagnostics for intestinal parasites. However, a notable exception is the FLOTAC technique.[48] This method can diagnose intestinal protozoa and helminth infections concurrently with high sensitivity,[49] and, in a recent study carried out in Côte d'Ivoire, its performance for hookworm diagnosis has been shown to be superior to the Kato–Katz and a standard SAF–ether–concentration method.[50] If the high sensitivity of this novel approach can be confirmed for other helminths and for intestinal protozoa, then it will offer a new means for comprehensive assessment of intestinal multiparasitism.

The increasing recognition of the common phenomenon of polyparasitism has yet to trigger additional research on how this influences treatment. While it is true that common anthelminthic drugs are active against a range of helminth species[51] and often exhibit some activity against additional parasites, there is no single drug to treat the full range of intestinal helminths. For intestinal protozoa, the situation is similar.[52] WHO recommends preventive chemotherapy without prior diagnosis in areas of high endemicity.[32,53] Clearly, nontarget parasites will be cotreated over the course of these campaigns, and it is possible that parasites are among them against which the drugs have never been tested. The practical evidence from the field suggests that most drugs do not exhibit high levels of adverse events, even if they are used in a context where multiparasitism has not been evaluated and where parasites other than the target species are likely to be present.[54] There are exceptions though, e.g., the administration of praziquantel to people suffering from cysticercosis.[55] Another area of concern is the development of resistance in nontarget parasites that could result from subtherapeutic drug concentrations. We are engaged in additional studies to investigate possible cross-benefits of chemotherapy resulting from the activity of anthelminthic drugs to nontarget parasites and we study the influence of polyparasitism on treatment outcomes. Finally, the results reported herein call for urgent interventions to reduce the burden of soil-transmitted helminthiasis, and the effect of regular administration of safe and efficacious anthelminthic drugs should be monitored.

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