

## Helminth infections and risk factor analysis among residents in Eryuan county, Yunnan province, China

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Received 9 February 2007; received in revised form 10 July 2007; accepted 11 July 2007

Available online 17 July 2007

### Abstract

Whilst infections with soil-transmitted helminths are common across China, the public-health significance of *Schistosoma japonicum* and food-borne helminths is more focalized. Only few studies have investigated the local epidemiology of helminth infections in rural China, including risk factor analysis. We collected stool and blood samples from 3220 individuals, aged 5–88 years, from 35 randomly selected villages in Eryuan county, Yunnan province, China. Stool samples were subjected to the Kato–Katz technique and examined for helminth eggs. Blood samples were tested for *Trichinella* spp., *S. japonicum* and cysticerci-specific antibodies. Data on individual and family-level risk factors were collected using questionnaires. The prevalence of *Ascaris lumbricoides*, *Taenia* spp., *Trichuris trichiura* and hookworms was 15.4%, 3.5%, 1.7% and 0.3%, respectively. The seroprevalence of *Trichinella* spp. was 58.8% and that of cysticercosis 18.5%. The egg positivity rate of *S. japonicum* in the 13 known endemic villages was 2.7%, and the corresponding seroprevalence was 49.5%. We observed a strong spatial heterogeneity in the families' economic status. *S. japonicum* infections were more prevalent among the Han than Bai nationality (odds ratio (OR) = 3.77, 95% confidence interval (CI) = 1.97–7.23) and tobacco growers (OR = 3.66, 95% CI = 1.77–7.60) and was only found at elevations below 2150 m above sea level. *A. lumbricoides* and *Taenia* spp. infections were more prevalent at altitudes above 2150 m when compared to lower settings (OR = 1.51, 95% CI = 1.24–1.84 and OR = 5.32, 95% CI = 3.42–8.28, respectively). The opposite was found for *T. trichiura* (OR = 0.31, 95% CI = 0.14–0.70). Our findings can guide the design and spatial targeting of control interventions against helminth infections in Eryuan county.

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**Keywords:** Soil-transmitted helminthiasis; Schistosomiasis; *Schistosoma japonicum*; *Taenia*; *Trichinella*; Cysticercosis; Risk factors; Multiparasitism; Socio-economic status; China

### 1. Introduction

Human helminth infections are common in China, particularly the soil-transmitted helminths, namely *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (mainly *Necator americanus*) (Mao, 1991; Xu

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et al., 1995). Until recently, it was believed that a substantial fraction of the global burden due to soil-transmitted helminthiasis is concentrated in China (de Silva et al., 2003). However, the epidemiology of many parasitic diseases has profoundly changed in the wake of China's rapid economic development (Banister and Zhang, 2005). In fact, recent surveys found significant declines in the prevalence of several helminths. Whilst the first nationwide survey carried out in the years 1988–1992 found prevalences of 47.0%, 18.8% and 17.2% for *A. lumbricoides*, *T. trichiura* and the hookworms, respectively (Xu et al., 1995), the second survey completed in 2004 indicated a drop in the respective prevalences to 12.7%, 4.6% and 6.1% (Ministry of Health, 2005). However, China's economic development exhibits a strong spatial heterogeneity, which in turn had an impact on the frequency of soil-transmitted helminths. Whilst in the late 1980s the eastern and southern provinces were affected most by soil-transmitted helminths (Xu et al., 1995), the highest prevalences are now observed in the central and western provinces, including Yunnan (Ministry of Health, 2005).

The only human pathogenic schistosome species in China is *Schistosoma japonicum*. Today, it still occurs in the marshlands and great lakes region along the Yangtze River in eastern China, and in mountainous areas of Sichuan and Yunnan. Sustained control activities implemented over the past 50 years brought down the total number of infections by over 90% (Utzinger et al., 2005; Zhou et al., 2005). The epidemiology of schistosomiasis japonica in eastern China has been studied in considerable detail but, thus far, few studies focused on the mountainous areas in the western part of the country (Spear et al., 2004). The reasons are multifactorial, including the complex local environment with small transmission foci, remoteness and lagging socio-economic development (Chen and Feng, 1999; Ross et al., 2001).

Food-borne trematode infections (e.g. *Clonorchis sinensis*) and other food-borne helminths (e.g. *Taenia* spp., *Trichinella* spp.) have a more patchy distribution linked to certain minorities and food consumption habits such as eating raw fish and raw meat. The local prevalence of these helminth species can be high and recent data suggest that they are emerging (Hotez et al., 1997; Lun et al., 2005; Ministry of Health, 2005; Cui et al., 2006). For Yunnan province, the third nationwide sampling survey on schistosomiasis carried out in 2004 estimated a prevalence of 1.7% in the 270 schistosome-endemic villages. Meanwhile, the second national parasitological survey reported a mean prevalence of 21.7% for soil-transmitted helminth infec-

tions (Ministry of Health, 2005). Antibodies against *Trichinella* spp. were found in 8.3% of the population; the highest rate in China.

The aim of the present study was to assess the prevalence of *S. japonicum*, soil-transmitted helminths and food-borne helminths using standardized parasitological and serological tests, and to investigate behavioural, demographic, environmental and socio-economic risk factors for infection using pre-tested questionnaires. Our geographical focus is on a partially schistosome-endemic county in Yunnan province.

## 2. Materials and methods

### 2.1. Study area and sample size

Eryuan county is situated in Dali prefecture, in the north-west of Yunnan province in southern China (25.80°–26.43°N latitude, 99.54°–100.34°E longitude, ~3000 km<sup>2</sup>). The county features two distinct landscapes: (i) fertile plains at an elevation of 1950–2150 m above sea level between Eryuan Lake and Erhai Lake; and (ii) mountain ranges with peaks of over 3000 m in the other regions. The mountain slopes are covered by fields or coniferous forests with a tree line at ~3000 m. The total population of Eryuan county was 273,000 in 2005; the majority of them belonging to the Bai ethnic group. Both Bai and the immigrated Han are primarily engaged in agriculture and animal husbandry. The intended sample size was 1% of the local residents, i.e. about 2700 people.

### 2.2. Population surveyed, informed consent and treatment

A 3.5 × 3.5 km-grid was laid over a map of the study area and 35 squares were randomly selected among the populated grid cells (Gyapong and Remme, 2001). If the cell contained two or more natural villages, one was randomly selected among those with at least 35 families. Subsequently, 35 families per community were randomly selected from the available village registries. Natural villages are spatial accumulations of houses within an administrative village; the lowest administrative level in China. All family members aged ≥5 years were included.

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). The leaders of the selected communities were invited to Eryuan city and informed about

the purpose and procedures of the study in the presence of leading administrative, medical and veterinary county officials. The objective, procedures, potential risks and benefits were explained and the village leaders were asked for their consent and support to conduct the study. Written informed consent was obtained from the heads of participating households. Those people tested positive for parasites in their stool were treated free of charge with appropriate drugs (i.e. praziquantel and/or albendazole) according to local and national standard treatment protocols (Li and Guan, 2004). Seropositives were informed and further testing was recommended.

### 2.3. Field procedures and questionnaires

The fieldwork was carried out in November/December 2005. Firstly, the selected families were contacted by a senior member of our research team. In case the head of household was absent or refused to participate, the neighbouring household was invited to participate. Secondly, a household questionnaire was administered to the head of the household. This questionnaire assessed house characteristics (e.g. building type and water supply), assets owned (e.g. TV and bicycle), and ownership of land and animals. Third, individual questionnaires were administered to all study participants aged  $\geq 15$  years. Younger participants answered the questionnaire with the assistance of their parents or legal guardians. Demographic information and data on raw food consumption and hand washing behaviour were collected. Both questionnaires were pre-tested in the study area with people who did not otherwise participate in the survey.

Fourth, the geographical coordinates of the participating households were recorded, using a hand-held global positioning system (GPS) receiver (Garmin Ltd., Olathe, USA). Finally, a venous blood sample of 3–5 ml was collected and labelled plastic bags for stool collection were handed out. Stool samples were either collected by fieldworkers or by a designated resident and sent to the local schistosomiasis control station in Eryuan city where they were kept in a cool place pending analysis.

### 2.4. Laboratory procedures

Four 42 mg Kato–Katz thick smears were prepared from each stool sample (Katz et al., 1972) and read by experienced technicians. The number of eggs of each parasite species was counted and recorded separately. A sub-sample of 5% of the slides was re-examined by the senior technician for quality control.

The serum samples were kept frozen and tested by enzyme-linked immunosorbent assay (ELISA) for IgG antibodies against *S. japonicum* (Shenzhen Combined Biotech Co. Ltd.; Shenzhen, China), cysticerci (larval stage of *T. solium*) and *Trichinella* spp. (both Hai Tai Co. Ltd.; Zhuhai, China), according to the manufacturer's instructions.

The sensitivity of the approach taken to detect *S. japonicum*-infections was assessed by collecting up to three stool specimens from all participants in three *S. japonicum*-endemic villages, which were then subjected to the Kato–Katz technique plus the hatching test (Justesen and van Sloterdijk, 1977).

### 2.5. Data management and statistical analysis

The questionnaire data were double entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). All further analyses were carried out in STATA version 9 (StataCorp LP; College Station, USA). The socio-economic status of the families was calculated according to an asset-based method put forward by HNP/World Bank (Gwatkin et al., 2000) and described in detail by Raso et al. (2005). In brief, asset weights were defined by principal component analysis (PCA) after replacement of missing values by the mean of the respective asset. With the exception of the number of cows and the area of irrigated agricultural land (measured in mǔ, a traditional Chinese surface unit; 1 mǔ = 666.7 m<sup>2</sup>), all assets had dichotomous character. The families were ranked into wealth quintiles according to their cumulative standardized asset scores.

The  $\chi^2$ -test was used to explore associations between the infection status and age and sex. Bivariate logistic regression was used to test for associations between infection and demographic variables, socio-economic status, village location, health-related behaviour and raw food consumption. Associations of infection status with hand washing behaviour and raw food consumption were further assessed by multiple logistic regression analysis. The models were adjusted for demographic variables, socio-economic status and village location whenever necessary (i.e.  $P < 0.1$  in bivariate analysis). Non-predicting covariates were removed at a level of  $P = 0.15$ , using a stepwise backward elimination procedure.

The final analysis only considered participants with complete questionnaire, parasitological and serological data. The family-level calculations were based on all available questionnaires, irrespective of stool and blood sample submission.

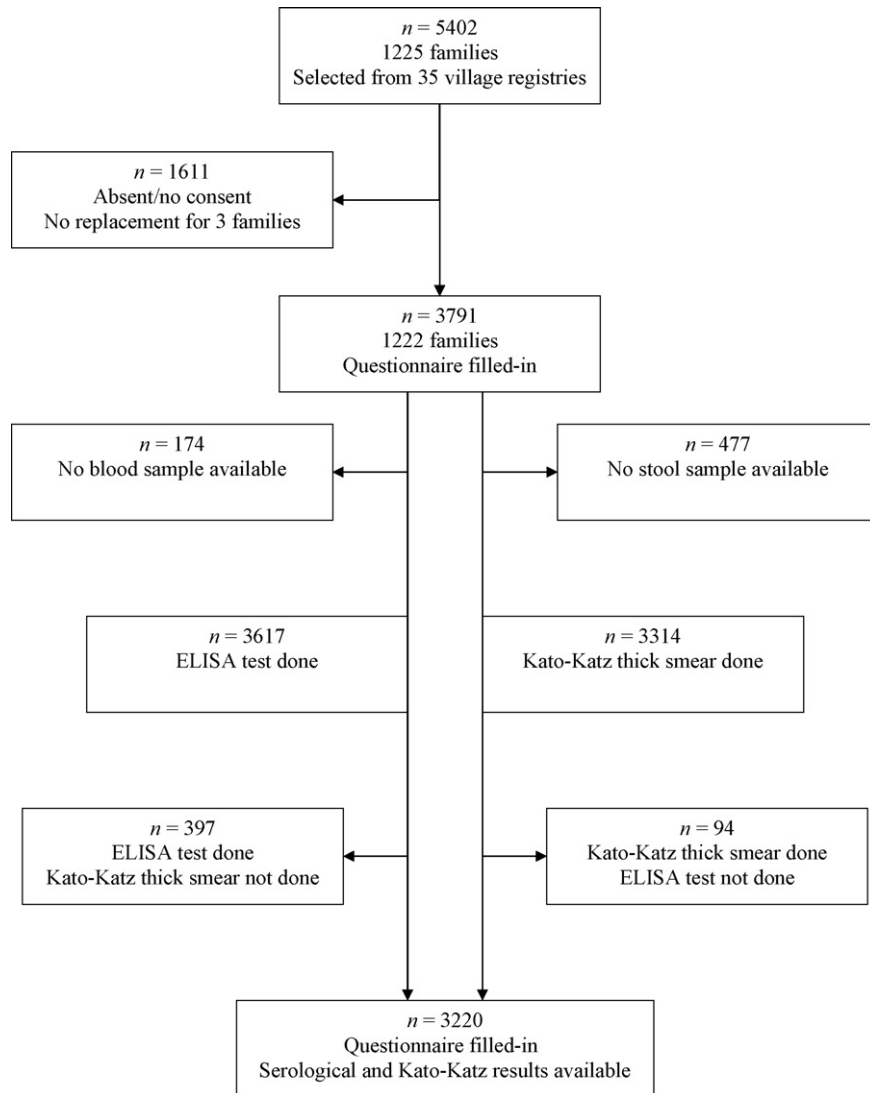


Fig. 1. Diagram detailing the study participation and compliance with blood and stool sample submission and being interviewed in 35 randomly selected villages in Eryuan county, Yunnan province, China. The final cohort comprised those with complete questionnaire, parasitological and serological data.

### 3. Results

#### 3.1. Study cohort, demographic and socio-economic profile

Of 5402 registered individuals in 1225 families selected for the study, 3791 people from 1222 families were present during our cross-sectional survey and answered the questionnaire (Fig. 1). Among them, 3617 (95.4%) consent to blood sampling and 3314 (87.4%) submitted a stool sample. Complete questionnaire data, blood and stool samples were available for 3220 individuals (84.9%). Compliance for stool and blood sample

provision varied between 45.6% and 96.6% among interviewed individuals at the unit of the village.

The study included 13 known schistosome-endemic villages that were home to 1429 individuals who had complete data records (44.4%). According to a local classification of village location, there were nine “plateau” villages, inhabited by 25.8% of the families, and 26 “mountain” villages, situated at 1750–2700 m. Most of the families (88.3%) lived in a 2-storey house with adobe walls and wooden load carrying parts.

From our final study cohort, 55.7% were females. The age structure was as follows: 5–9 years (8.6%), 10–14 years (7.7%), 15–24 years (12.9%), 25–39 years (35.0%)

and  $\geq 40$  years (35.8%). There was no statistically significant difference in the number of males and females in the different age groups ( $\chi^2 = 5.83$ , degree of freedom (d.f.) = 4,  $P = 0.212$ ). Bai was the dominant ethnic group (80.3%) and Han Chinese accounted for the remaining 19.7%. While Bai lived in plain and mountainous regions, Han predominantly lived in the fertile and economically more advanced plain areas. Most participants were farmers (79.7%) or students (15.8%). Only 5.0% reported significant non-agricultural sources of income. Domestic animals were kept by 95.9% of the families. Pigs and dogs were particularly common (78.4% and 67.6%, respectively). Cattle were owned by 37.6% and cows by 26.8% of the families, the former only in mountain villages and the latter predominantly in plain areas. Other animals included goats (19.5%), mules (16.7%), horses (9.2%), buffaloes (9.2%), sheep (4.6%) and donkeys (2.0%).

The first principal component of the model used to assess the socio-economic status of the families explained 29.7% of the total variability. As shown in Table 1, the greatest weights were attached to households possessing a bicycle (0.35) or an electric rice cooker (0.34). Owning a black/white TV had the lowest weight (0.09). Standardization of the asset weights resulted in greatest weights being attached to cars (0.90) and motorbikes (0.89). Lowest scores resulted from the absence of a colour TV (−0.33). Every m<sup>2</sup> of irrigated land or cow increased the score of a household by 0.32 or 0.14, respectively. None of the assets investigated were owned by the poorest families.

Common assets were irrigated land (62.0%) and a colour TV (54.5%). Assets owned by more than 90% of the least poor families included irrigated land or a colour TV (both 97.1%), and an electric rice cooker (90.2%). Among the very poor households, 58.2% owned irrigated land, 19.0% a black/white TV and 13.9% a radio. Only 9.9% of the households were not connected to the power grid, but in the lowest quintile, electricity was not available in 39.9% of the households (data not presented).

### 3.2. Helminth infections

The results from our cross-sectional parasitological and serological survey, stratified by sex and age, are summarized in Table 2. The overall prevalence of *A. lumbricoides* infection was 15.4% and showed large village-to-village variation (0.8–48.0%). The prevalence of *Taenia solium*/*T. saginata* infection was 3.5% (range: 0–19.1%), that of *T. trichiura* 1.7% (range: 0–26.7%) and that of hookworms 0.3% (only found in 9 villages). *S. japonicum* eggs were found in 1.3% of all participants.

The prevalence of *S. japonicum* in the 13 known endemic villages was 2.7% (range: 0–11.6%). No statistically significant difference was observed in the prevalence of any parasite by either sex or age ( $P > 0.05$ ). Overall, helminth eggs were found in the stools of 20.5% of all participants; 18.8% of the cohort was infected by a single species, whereas dual (1.6%) and triple infections (0.1%) were rare.

Antibodies against *Trichinella* spp. and cysticerci were found in 58.8% and 18.5% of the blood samples, respectively, and the schistosomiasis seroprevalence in the 13 known endemic villages was 49.5%. The seroprevalence of trichinellosis was significantly higher in males than in females ( $P < 0.001$ ), and increased with age ( $P < 0.001$ ). The schistosomiasis seroprevalence was higher in females when compared to males ( $P = 0.002$ ) and reached the highest level in the 25–39 year age group ( $P < 0.001$ ).

Significant associations were found between infections with different parasites or serostatus. *A. lumbricoides* infection was associated with *T. trichiura* (odds ratio (OR) = 4.03,  $P < 0.001$ ), trichinellosis seropositivity (OR = 0.64,  $P < 0.001$ ) and *S. japonicum* seropositivity among all study participants (OR = 0.54,  $P < 0.001$ ). *Taenia* spp. eggs were found more often in the stools of those with positive cysticercosis serology (OR = 4.59,  $P < 0.001$ ), but were less frequent among *S. japonicum* seropositives in the whole study cohort (OR = 0.49,  $P = 0.007$ ). Eggs of *S. japonicum* were found more often among cysticercosis seropositives in schistosome-endemic villages (OR = 2.14,  $P = 0.043$ ). Trichinellosis seropositivity was associated with the detection of *S. japonicum* eggs and antibodies in the whole study area and in *S. japonicum*-endemic villages only (OR = 4.21–9.30).

### 3.3. Ethnicity, educational attainment and occupation

Tables 3 and 4 show that among the participants aged 18 years and above, illiterates were more likely to be infected with *A. lumbricoides* than those with basic education (OR = 0.76,  $P = 0.023$ ) or higher education (OR = 0.47,  $P = 0.027$ ). *S. japonicum* infection, as assessed by the Kato–Katz technique, was more prevalent among Han than Bai (5.8% versus 1.6%; OR = 3.77,  $P < 0.001$ ). The former ethnic group was also more likely to have antibodies against this parasite (OR = 2.40,  $P < 0.001$ ), as well as against *Trichinella* spp. (OR = 2.51,  $P < 0.001$ ). Tobacco growing was another risk factor for *S. japonicum* infection (7.0% versus 2.0%; OR = 3.66,  $P < 0.001$ ) and seropositivity (OR = 4.32,  $P < 0.001$ ). In

Table 1

Household assets considered for the calculation of the socio-economic status of the families, their factor scores, score of household if asset present or absent and asset distribution among families, stratified into five wealth quintiles

Asset	Asset factor score	Household score asset present	Household score asset absent	No.	Household have asset <i>n</i> (%)	1st quintile Most poor <i>n</i> (%) [total = 253]	2nd quintile Very poor <i>n</i> (%) [total = 237]	3rd quintile Poor <i>n</i> (%) [total = 244]	4th quintile Less poor <i>n</i> (%) [total = 244]	5th quintile Least poor <i>n</i> (%) [total = 244]
Telephone	0.309	0.60	−0.16		257 (21.0)	0 (0)	2 (0.8)	23 (9.4)	60 (24.6)	172 (70.5)
Radio	0.157	0.37	−0.07		183 (15.0)	0 (0)	33 (13.9)	24 (9.8)	38 (15.6)	88 (36.1)
Black/white TV	0.087	0.18	−0.04		237 (19.4)	0 (0)	45 (19.0)	62 (25.4)	62 (25.4)	68 (27.8)
Colour TV	0.305	0.28	−0.33		666 (54.5)	0 (0)	67 (28.3)	163 (66.8)	199 (81.6)	237 (97.1)
VCD player <sup>a</sup>	0.308	0.45	−0.21		393 (32.2)	0 (0)	1 (0.4)	81 (33.2)	114 (46.7)	197 (80.7)
Electric fan	0.203	0.53	−0.08		156 (12.8)	0 (0)	3 (1.3)	17 (7.0)	54 (22.1)	82 (33.6)
Electric rice cooker	0.336	0.42	−0.27		471 (38.5)	0 (0)	3 (1.3)	80 (32.8)	168 (68.9)	220 (90.2)
Washing machine	0.318	0.71	−0.14		204 (16.7)	0 (0)	0 (0)	1 (0.4)	46 (18.9)	157 (64.3)
Refrigerator	0.236	0.86	−0.06		85 (7.0)	0 (0)	0 (0)	0 (0)	13 (5.3)	72 (29.5)
Bicycle	0.345	0.53	−0.23		367 (30.0)	0 (0)	0 (0)	31 (12.7)	130 (53.3)	206 (84.3)
Motorbike	0.238	0.89	−0.06		81 (6.6)	0 (0)	0 (0)	2 (0.8)	7 (2.9)	72 (29.5)
Tractor	0.192	0.70	−0.05		85 (7.0)	0 (0)	0 (0)	5 (2.1)	14 (5.7)	66 (27.1)
Car	0.100	0.90	−0.01		15 (1.2)	0 (0)	0 (0)	2 (0.8)	1 (0.4)	12 (4.9)
No. of cows owned	0.289	1: 0.17+0.32/cow	−0.16	0	894 (72.2)	253 (100)	228 (96.2)	203 (83.2)	135 (55.3)	75 (30.7)
				1	149 (12.2)	0 (0)	9 (3.8)	23 (9.4)	63 (25.8)	54 (22.1)
				≥2	179 (14.6)	0 (0)	0 (0)	18 (7.4)	46 (18.9)	115 (47.1)
Mū irrigated land for agriculture <sup>b</sup>	0.259	1: −0.09 +0.14/mū	−0.23	0	464 (38.0)	253 (100)	99 (41.8)	81 (33.2)	24 (9.8)	7 (2.9)
				0.1–1.9	255 (20.9)	0 (0)	79 (33.3)	58 (23.8)	65 (26.6)	53 (21.7)
				2.0–3.9	350 (28.7)	0 (0)	50 (21.1)	76 (31.2)	105 (43.0)	119 (48.8)
				≥4.0	153 (12.4)	0 (0)	9 (3.8)	29 (11.9)	50 (20.5)	65 (26.6)

<sup>a</sup> VCD: video compact disc.

<sup>b</sup> 1 mū = 666.7 m<sup>2</sup>.



Table 2

Prevalence of parasitic infections diagnosed by the Kato–Katz technique and seroprevalence assessed by ELISA in 35 randomly selected villages in Eryuan county, Yunnan province, China. Data are stratified by sex and age

Parasite	Prevalence (95% CI)	Sex				Age (years)						
		F	M	$\chi^2$	<i>P</i>	5–9	10–14	15–24	25–39	≥40	$\chi^2$	<i>P</i>
Eggs detected												
<i>Ascaris lumbricoides</i> <sup>a</sup>	15.4 (14.2–16.7)	16.3	14.3	2.41	0.120	19.9	18.6	14.4	14.6	14.8	7.54	0.110
<i>Taenia</i> spp. <sup>a</sup>	3.5 (2.9–4.2)	3.2	4.0	1.55	0.214	1.5	3.6	3.8	4.0	3.5	4.35	0.360
<i>Trichuris trichiura</i> <sup>a</sup>	1.7 (1.2–2.1)	1.6	1.7	0.02	0.886	1.1	3.2	1.2	1.1	2.2	9.19	0.056
<i>Schistosoma japonicum</i> <sup>a</sup>	1.3 (0.9–1.8)	1.4	1.2	0.25	0.614	0.7	2.8	0.7	1.2	1.5	6.77	0.149
<i>Schistosoma japonicum</i> <sup>b</sup>	2.7 (1.9–3.6)	2.7	2.7	<0.01	0.984	1.7	6.2	1.9	2.4	2.6	6.45	0.168
Infection with any helminth <sup>a</sup>	20.5 (19.1–21.9)	21.1	19.8	0.85	0.357	22.1	26.3	18.7	19.7	20.3	6.85	0.144
Antibodies detected												
<i>Schistosoma japonicum</i> ELISA <sup>a</sup>	27.1 (25.6–28.7)	29.2	24.5	8.93	0.003	11.2	20.7	22.3	31.8	29.4	60.85	<0.001
<i>Schistosoma japonicum</i> ELISA <sup>b</sup>	49.5 (46.9–52.1)	53.2	44.9	8.47	0.002	22.3	40.7	48.2	58.0	50.0	53.68	<0.001
Cysticercosis ELISA <sup>a</sup>	18.5 (17.2–19.9)	17.1	20.3	7.13	0.018	13.8	18.2	17.5	19.2	19.4	5.37	0.252
<i>Trichinella</i> spp. ELISA <sup>a</sup>	58.8 (57.0–60.5)	55.4	63.0	24.48	<0.001	24.6	44.1	48.0	63.5	69.3	238.07	<0.001

<sup>a</sup> All villages (*n* = 3220 individuals).

<sup>b</sup> In the 13 known *S. japonicum*-endemic villages (*n* = 1429 individuals).

addition, growing tobacco was positively associated with cysticercosis (OR = 1.57, *P* < 0.001) and trichinellosis seropositivity (OR = 1.98, *P* < 0.001).

### 3.4. Socio-economy, environment and behaviour

Eryuan county is geographically and economically heterogeneous. The spatial variation of the socio-economic composition in the random sample of villages considered here is depicted in Fig. 2. The proportion of lower strata was higher in the western mountains. Only 1.8% of the poorest and 96.6% of the least poor families lived at an altitude <2150 m. None of the poorest and only 1.7% of the very poor families lived in plateau villages where 69.3% of the least poor families lived. Families of all five socio-economic strata were found in only five villages, and all families of one village fell into the ‘most poor’ category. Members of poor, less poor and least poor families were significantly less likely to be infected with *A. lumbricoides* or *Taenia* spp. than the poorest (Table 3). Table 4 shows that in known schistosome-endemic villages, members of less and least poor families were less likely to be *S. japonicum* seropositive (OR = 0.54, *P* = 0.004 and OR = 0.48, *P* = 0.001, respectively). The lowest *Trichinella* spp. antibody seroprevalence was found among the most poor.

Living at an elevation ≥2150 m was a significant risk factor for *A. lumbricoides* (OR = 1.51, *P* < 0.001) and *Taenia* spp. (OR = 5.32, *P* < 0.001), but was protective against an infection with *T. trichiura* (OR = 0.31, *P* = 0.005). *S. japonicum* infections were only found below 2150 m (Table 3). Antibodies against cysticerci

were associated with lower education, residency at ≥2150 m or in a mountain village, and low socio-economic status (Table 4). The same characteristics were protective factors for trichinellosis.

Reported hand washing before meals and after defecation were significantly associated with each other, with higher socio-economic status (quintiles 3–5) and, negatively, with living in a mountain village or in villages situated at an altitude ≥2150 m (all *P* < 0.001, data not shown).

The associations between infection status and hand washing and food consumption are summarized in Table 5. Washing hands before meals was protective against *A. lumbricoides* (OR = 0.74, *P* = 0.011) and hand washing after defecation was a negative predictor for cysticercosis seropositivity (OR = 0.77, *P* = 0.015). The consumption of raw pork was a positive predictor for *Trichinella* spp. seropositivity (OR = 1.56, *P* = 0.002).

## 4. Discussion

There is a paucity of community-based studies assessing the prevalence of helminth infections and underlying risk factors in rural China and Southeast Asia more generally (Ohta and Waikagul, 2006). We carried out a study in 35 randomly selected villages in the mountainous county of Eryuan, located in the Yunnan province, which is partially endemic for *S. japonicum*. The analysis of a single stool sample by the Kato–Katz technique resulted in low mean prevalences of soil-transmitted helminths, *S. japonicum* and *Taenia* spp. However, a single blood sample subjected to an ELISA test resulted in high

Table 3

Bivariate logistic regression analyses of the relationship between the infection risk with different helminths and demographic indicators, hand washing behaviour, raw food consumption, village location and socio-economic status in Eryuan county, Yunnan province, China

Risk factor	<i>Ascaris lumbricoides</i> <sup>a</sup>		<i>Taenia</i> spp. <sup>a</sup>		<i>Trichuris trichiura</i> <sup>a</sup>		<i>Schistosoma japonicum</i> <sup>b</sup>	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Sex								
Female	1.00		1.00		1.00		1.00	
Male	0.86 (0.71–1.04)	0.120	1.27 (0.87–1.84)	0.215	1.04 (0.60–1.80)	0.886	1.01 (0.53–1.92)	0.984
Age (years)								
5–9	1.00		1.00		1.00		1.00	
10–14	0.92 (0.59–1.42)	0.706	2.57 (0.78–8.46)	0.120	3.05 (0.80–11.61)	0.103	3.93 (0.80–19.33)	0.092
15–24	0.68 (0.45–1.01)	0.056	2.71 (0.90–8.20)	0.077	1.10 (0.26–4.66)	0.892	1.12 (0.18–6.82)	0.090
25–39	0.69 (0.49–0.96)	0.029	2.83 (1.01–7.94)	0.048	0.98 (0.27–3.50)	0.975	1.48 (0.33–6.69)	0.612
≥40	0.70 (0.50–0.98)	0.037	2.44 (0.87–6.88)	0.091	2.02 (0.60–6.72)	0.254	1.59 (0.36–7.09)	0.543
Education <sup>c</sup>								
Illiterate	1.00		1.00		1.00		1.00	
≤Junior middle school	0.76 (0.61–0.96)	0.023	0.71 (0.47–1.08)	0.110	1.20 (0.60–2.36)	0.607	0.95 (0.35–2.58)	0.925
≥High middle school	0.47 (0.24–0.92)	0.027	0.18 (0.02–1.32)	0.091	n.a.	n.a.	2.04 (0.47–8.76)	0.340
Ethnic group								
Bai	1.00		1.00		1.00		1.00	
Han	0.93 (0.73–1.19)	0.570	0.82 (0.49–1.34)	0.423	0.95 (0.48–1.91)	0.893	3.77 (1.97–7.23)	<0.001
Socio-economic status								
Most poor	1.00		1.00		1.00		1.00	
Very poor	0.80 (0.59–1.08)	0.143	0.78 (0.48–1.27)	0.323	3.73 (1.05–13.29)	0.042	n.a.	n.a.
Poor	0.61 (0.45–0.82)	0.001	0.55 (0.33–0.93)	0.024	4.28 (1.23–14.86)	0.022	0.72 (0.16–3.29)	0.674
Less poor	0.56 (0.41–0.75)	<0.001	0.27 (0.14–0.50)	<0.001	3.00 (0.84–10.70)	0.090	0.86 (0.24–3.13)	0.817
Least poor	0.51 (0.38–0.68)	<0.001	0.10 (0.04–0.24)	<0.001	2.44 (0.68–8.78)	0.173	1.21 (0.35–4.13)	0.765
Livestock breeder	0.54 (0.44–0.67)	<0.001	0.90 (0.61–1.32)	0.573	0.46 (0.25–0.87)	0.017	0.76 (0.39–1.46)	0.406
Tobacco grower	0.65 (0.45–0.93)	0.019	1.24 (0.70–2.20)	0.454	0.53 (0.16–1.71)	0.288	3.66 (1.77–7.60)	<0.001
Temporary employment	1.65 (0.98–2.78)	0.060	1.39 (0.50–3.87)	0.526	1.51 (0.36–6.31)	0.573	n.a.	n.a.
Resident at altitude ≥2150 m	1.51 (1.24–1.84)	<0.001	5.32 (3.42–8.28)	<0.001	0.31 (0.14–0.70)	0.005	n.a.	n.a.
Village location								
Plain area	1.00		1.00		1.00		1.00	
Mountains	1.43 (1.16–1.77)	0.001	5.39 (2.80–10.35)	<0.001	1.15 (0.64–2.08)	0.636	0.73 (0.35–1.52)	0.403
Washing hands								
Before meals	0.89 (0.75–1.07)	0.227	0.45 (0.29–0.70)	<0.001	1.09 (0.72–1.65)	0.677	0.83 (0.44–1.59)	0.581
After defecation	0.95 (0.82–1.11)	0.517	0.49 (0.32–0.75)	0.001	1.05 (0.73–1.52)	0.795	0.71 (0.37–1.34)	0.285
Food consumption								
Raw pork	0.52 (0.40–0.69)	<0.001	0.86 (0.48–1.55)	0.611	0.27 (0.15–0.50)	<0.001	3.17 (0.43–23.37)	0.257
Raw beef	0.84 (0.67–1.05)	0.122	0.46 (0.27–0.79)	0.005	1.81 (1.03–3.18)	0.039	0.50 (0.23–1.11)	0.088
Raw fish	1.37 (0.76–2.48)	0.294	0.38 (0.05–2.79)	0.343	0.86 (0.12–6.31)	0.881	3.22 (0.73–14.16)	0.122
Raw vegetables	0.84 (0.52–1.34)	0.456	2.29 (0.56–9.40)	0.248	0.48 (0.17–1.36)	0.169	1.82 (0.24–13.44)	0.559
Raw water plants	0.77 (0.64–0.93)	0.008	0.40 (0.27–0.60)	<0.001	0.66 (0.38–1.14)	0.138	0.56 (0.29–1.07)	0.078

OR: odds ratio; CI: confidence interval; P: based on likelihood ratio test; n.a.: not applicable.

<sup>a</sup> All villages (n = 3220 individuals).

<sup>b</sup> In the 13 known *S. japonicum*-endemic villages (n = 1429 individuals).

<sup>c</sup> Among participants aged ≥18 years (n = 2549; n = 1131 in the 13 known *S. japonicum*-endemic villages).



Table 4

Bivariate logistic regression analyses of the relationship between the serostatus and demographic indicators, hand washing behaviour, raw food consumption, village location and socio-economic status in Eryuan county, Yunnan province, China

Risk factor	<i>Schistosoma japonicum</i> ELISA <sup>a</sup>		Cysticercosis ELISA <sup>b</sup>		<i>Trichinella</i> spp. ELISA <sup>b</sup>	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Sex						
Female	1.00		1.00		1.00	
Male	0.72 (0.58–0.89)	0.002	1.24 (1.04–1.48)	0.018	1.37 (1.19–1.58)	<0.001
Age (years)						
5–9	1.00		1.00		1.00	
10–14	2.39 (1.35–4.22)	0.003	1.40 (0.87–2.23)	0.166	2.42 (1.67–3.50)	<0.001
15–24	3.23 (1.91–5.48)	<0.001	1.33 (0.87–2.03)	0.190	2.82 (2.02–3.94)	<0.001
25–39	4.80 (3.02–7.64)	<0.001	1.49 (1.02–2.16)	0.037	5.32 (3.94–7.18)	<0.001
≥40	3.48 (2.20–5.52)	<0.001	1.51 (1.04–2.19)	0.030	6.91 (5.11–9.34)	<0.001
Education <sup>c</sup>						
Illiterate	1.00		1.00		1.00	
≤Junior middle school	1.10 (0.81–1.49)	0.533	0.71 (0.58–0.88)	0.001	1.96 (1.64–2.33)	<0.001
≥High middle school	0.65 (0.37–1.15)	0.137	0.55 (0.32–0.97)	0.037	2.37 (1.53–3.68)	<0.001
Ethnic group						
Bai	1.00		1.00		1.00	
Han	2.40 (1.87–3.07)	<0.001	1.07 (0.86–1.34)	0.536	2.51 (2.06–3.05)	<0.001
Socio-economic status						
Most poor	1.00		1.00		1.00	
Very poor	n.a.		0.98 (0.73–1.32)	0.913	2.48 (1.94–3.17)	<0.001
Poor	0.71 (0.44–1.14)	0.158	1.20 (0.91–1.60)	0.197	2.34 (1.84–2.97)	<0.001
Less poor	0.54 (0.35–0.82)	0.004	0.82 (0.61–1.09)	0.168	3.77 (2.97–4.78)	<0.001
Least poor	0.48 (0.32–0.73)	0.001	0.63 (0.47–0.84)	0.002	4.70 (3.71–5.96)	<0.001
Livestock breeder	1.20 (0.97–1.48)	0.090	1.02 (0.85–1.23)	0.802	1.89 (1.63–2.19)	<0.001
Tobacco grower	4.32 (2.91–6.41)	<0.001	1.57 (1.20–2.05)	0.001	1.98 (1.53–2.55)	<0.001
Temporary employment	1.44 (0.63–3.26)	0.383	1.22 (0.72–2.08)	0.453	1.06 (0.68–1.66)	0.788
Resident at altitude ≥2150 m	n.a.		1.63 (1.36–1.96)	<0.001	0.47 (0.41–0.55)	<0.001
Village location						
Plain area	1.00		1.00		1.00	
Mountains	1.02 (0.82–1.27)	0.870	1.73 (1.41–2.12)	<0.001	0.37 (0.32–0.43)	<0.001
Washing hands						
Before meals	0.88 (0.72–1.09)	0.239	0.78 (0.65–0.93)	0.006	1.32 (1.15–1.52)	<0.001
After defecation	1.01 (0.86–1.19)	0.926	0.75 (0.63–0.89)	0.001	1.18 (1.05–1.33)	0.006
Food consumption						
Raw pork	1.25 (0.85–1.84)	0.261	1.47 (1.06–2.05)	0.023	1.38 (1.09–1.74)	0.007
Raw beef	0.77 (0.62–0.92)	0.088	0.92 (0.74–1.13)	0.409	1.49 (1.26–1.76)	<0.001
Raw fish	1.20 (0.55–2.61)	0.647	0.90 (0.48–1.68)	0.731	1.82 (1.08–3.07)	0.025
Raw vegetables	1.44 (0.87–2.37)	0.158	1.27 (0.77–2.08)	0.352	0.67 (0.46–0.98)	0.040
Raw water plants	0.86 (0.70–1.07)	0.180	0.93 (0.78–1.11)	0.445	1.09 (0.95–1.25)	0.235

OR: odds ratio; CI: confidence interval; P: based on likelihood ratio test; n.a.: not applicable.

<sup>a</sup> In the 13 known *S. japonicum*-endemic villages (*n* = 1429 individuals).

<sup>b</sup> All villages (*n* = 3220 individuals).

<sup>c</sup> Among participants aged ≥18 years (*n* = 2549; *n* = 1131 in known *S. japonicum*-endemic villages).

seroprevalences of schistosomiasis japonica, trichinellosis and cysticercosis. Risk factors for infection were determined by bivariate and multiple logistic regression analysis.

Our final study cohort (*n* = 3220) exceeded the estimated minimum sample size by approximately 20%. It

included a higher proportion of females and the average age was higher than that of the population initially selected from the community registries. We speculate that the difference arises from out-migration of primarily young males for education and labour. It must also be considered that Chinese villagers usually remain reg-

Table 5

Stepwise multiple logistic regression to assess associations between hand washing behaviour, raw food consumption and parasite infection status

Risk factors	<i>A. lumbricoides</i> <sup>a</sup>		<i>Taenia</i> spp. <sup>a</sup>		<i>T. trichiura</i> <sup>a</sup>		<i>S. japonicum</i> <sup>b</sup>		<i>S. japonicum</i> ELISA <sup>b</sup>		Cysticercosis ELISA <sup>a</sup>		<i>Trichinella</i> spp. ELISA	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Washing hands														
Before meals	0.74 (0.58–0.93)	0.011	0.67 (0.42–1.08)	0.103	0.46 (0.23–0.92)	0.028	*	*	*	*	1.17 (0.98–1.41)	0.085		
After defecation	*		*		*		*	*	0.77 (0.63–0.95)	0.015	*			
Consumption of														
Raw pork	0.51 (0.37–0.69)	<0.001	*		0.34 (0.16–0.73)	0.006	*	*	1.32 (0.93–1.96)	0.119	1.56 (1.18–2.05)	0.002		
Raw beef	*		*		2.17 (1.13–4.16)	0.019	*	0.34 (0.14–0.81)	0.014	0.72 (0.56–0.93)	0.011	0.78 (0.64–0.96)	0.019	
Raw vegetables	*		*		0.44 (0.14–1.33)	0.145	*	*	*	*	0.57 (0.36–0.90)	0.015		
Raw fish	*		*		*		4.57 (0.95–22.10)	0.059	*	*	*			
Raw water plants	0.79 (0.64–0.98)	0.033	0.54 (0.35–0.83)	0.005	*		*	*	*	*	0.78 (0.66–0.93)	0.006		
Sex														
Female	**		**		**		**		1.00		1.00		1.00	
Male	**		**		**		**		0.70 (0.56–0.88)	0.003	1.24 (1.03–1.50)	0.023	1.45 (1.24–1.71)	<0.001
Age (years)														
5–9	*		*		**		*		1.00		*		1.00	
10–14	*		*		**		*		2.03 (1.09–3.77)	0.025	*		2.37 (1.57–3.56)	<0.001
15–24	*		*		**		*		3.36 (1.90–5.94)	<0.001	*		3.38 (2.34–4.88)	<0.001
25–39	*		*		**		*		4.86 (2.93–8.06)	<0.001	*		6.25 (4.49–8.69)	<0.001
≥40	*		*		**		*		4.01 (2.43–6.62)	<0.001	*		9.21 (6.60–12.87)	<0.001
Ethnic group														
Bai	**		**		**		1.00		1.00		**		1.00	
Han	**		**		**		4.25 (2.13–8.45)	<0.001	2.33 (1.74–3.22)	<0.001	**		1.99 (1.57–2.52)	<0.001
Livestock breeder	0.56 (0.44–0.70)	<0.001	**		0.45 (0.22–0.90)	0.025	**		*		**		*	
Tobacco grower	*		**		**		*		2.65 (1.68–4.19)	<0.001	1.52 (1.13–2.04)	0.006	*	
Temporary employment	1.71 (0.98–2.98)	0.059	**		**		**		**		**		**	
Resident at altitude ≥2150 m	*		4.19 (2.64–6.66)	<0.001	0.32 (0.14–0.74)	0.008	n.a.		n.a.		1.37 (1.04–1.80)	0.027	*	
Socio-economic status														
Most poor	1.00		*		*		**		1.00		1.00		1.00	
Very poor	0.76 (0.56–1.05)	0.093	*		*		**		1.58 (0.95–2.62)	0.078	1.16 (0.83–1.61)	0.392	2.71 (2.06–3.55)	<0.001
Poor	0.55 (0.39–0.77)	<0.001	*		*		**		n.a.		1.56 (1.11–2.19)	0.010	2.05 (1.56–2.69)	<0.001
Less poor	0.51 (0.35–0.73)	<0.001	*		*		**		0.68 (0.48–0.98)	0.037	1.40 (0.94–2.08)	0.099	2.64 (1.97–3.55)	<0.001
Least poor	0.51 (0.34–0.75)	0.001	*		*		**		0.51 (0.36–0.730)	<0.001	1.28 (0.84–1.96)	0.249	2.76 (2.01–3.79)	<0.001
Village location														
Plain area	1.00		*		**		**		**		1.00		1.00	
Mountains	0.76 (0.56–1.03)	0.079	*		**		**		**		1.46 (1.10–1.93)	0.68	0.54 (0.43–0.68)	<0.001

Analyses have been adjusted for demographic indicators, village location and socio-economic status whenever necessary ( $P < 0.1$  in bivariate analysis). OR: odds ratio, CI: confidence interval,  $P$ : based on likelihood ratio test, n.a.: not applicable.

<sup>a</sup> All villages ( $n = 3220$  individuals).

<sup>b</sup> In the 13 known *S. japonicum*-endemic villages ( $n = 1429$  individuals).

\* Removed at a level of  $P = 0.15$ .

\*\*  $P \geq 0.1$  in bivariate analysis.

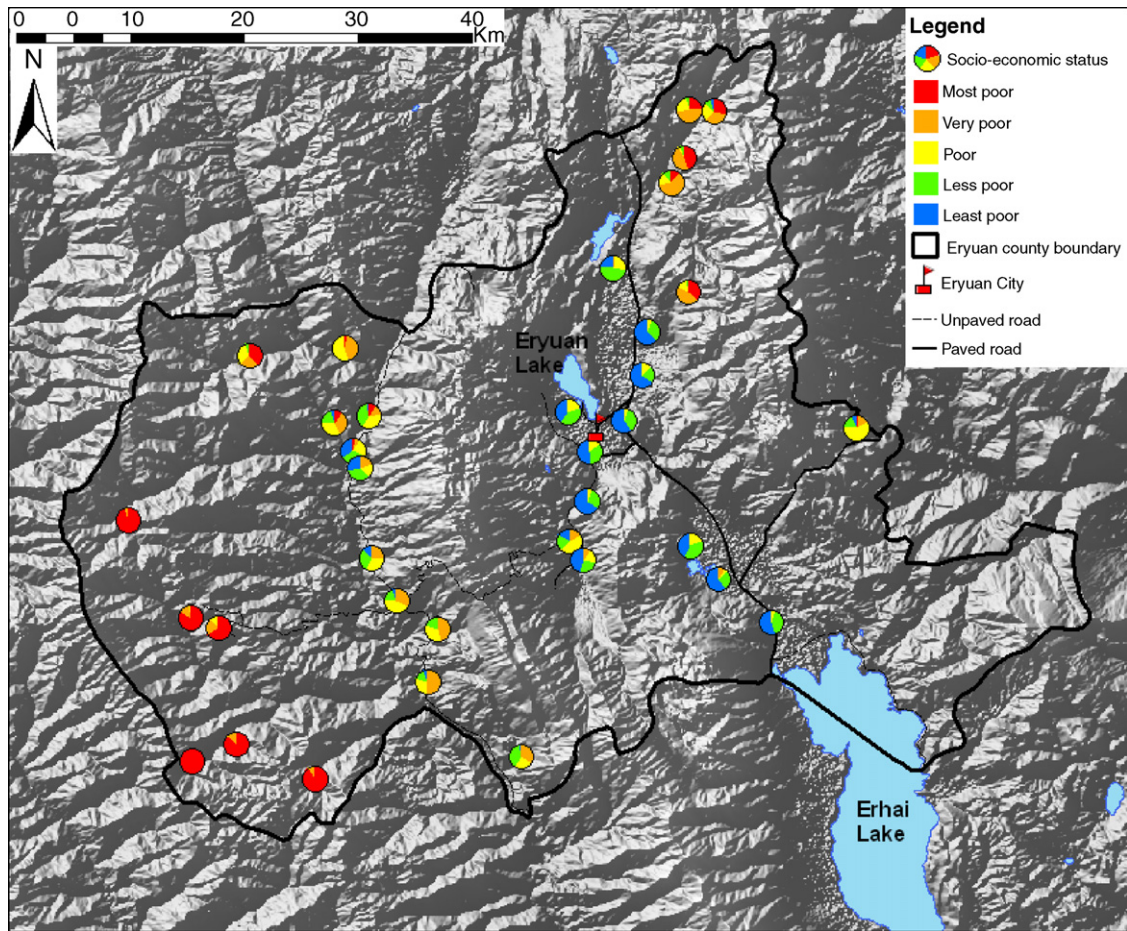


Fig. 2. Physical relief of Eryuan county, Yunnan province, China, based on data downloaded from the Shuttle Radar Topography Mission, U.S. Geological Survey website (<http://www.usgs.gov/>). The location of the study villages and the respective wealth quintile distribution among the participating families are depicted. Also shown is the road network.

istered in their native village even if they spend most of their time outside the county. Therefore, we assume that our sample is nevertheless representative of the actual resident population.

Possible reasons for the low prevalence of soil-transmitted helminth infections include the climate and recent economic advances, which improved access to clean water, sanitation and anthelmintic drugs (de Silva et al., 2003; Bethony et al., 2006). The prevalence of *A. lumbricoides* was higher among the poor and in those with no or only basic education. The higher prevalence observed in villages located  $\geq 2150$  m and in mountain villages probably reflects the generally lower socio-economic status in those areas. A certain fraction of the *A. lumbricoides* infections might also have been acquired elsewhere as the prevalence was especially high in those reporting temporary employment. *T. trichiura* was almost exclusively found at elevations  $< 2150$  m. The

observed hookworm prevalence was 0.3%. The reason for the low prevalence of hookworm infection could be the cool climate. However, the time delays between stool production by study participants and sample collection by our team and transportation to the laboratory, and loss of hookworm eggs due to time lags of up to several hours between the Kato–Katz thick smear preparation and examination under a microscope could be other reasons (Martin and Beaver, 1968; Dacombe et al., 2007).

*Taenia* spp. infections and cysticercosis seropositivity were particularly prominent in the poor with no or only basic education, living at altitudes  $\geq 2150$  m. Trichinellosis seropositivity, on the other hand, was more prevalent at lower altitudes, among elderly men, the more affluent and the Han nationality.

The measured seroprevalence rates for schistosomiasis japonica, cysticercosis and especially trichinellosis are high, but we are confident that they accurately rep-

resent the local conditions. This assumption is based on the following grounds. Firstly, we used standard tests recommended by the National Institute of Parasitic Diseases in Shanghai. Secondly, the common exposition to risk factors (water contact, low hygiene conditions, raw meat consumption) favours high seroconversion rates. Trichinellosis outbreaks and cysticercosis cases are common in Dali prefecture and Eryuan is the most severely affected county. In the case of the ELISA test for *Trichinella* spp., cross-reactions are known to occur with antibodies against *Toxoplasma* spp., *Echinococcus* spp. and cysticerci. In our study, we found no significant association between cysticercosis and trichinellosis seropositivity ( $\chi^2 = 0.12$ ,  $P = 0.726$ ).

Tobacco farming was reported by 35.2% of the Han but only 4.3% of the Bai people. Both tobacco growing and being Han were the most prominent risk factors for an infection with *S. japonicum*. Similar results were reported from an endemic area in Sichuan province by Spear et al. (2004) who found that villages devoting a higher percentage of their land to tobacco and vegetable growing had higher prevalences. It is also conceivable that some of the infections were imported. The prevalence of *S. japonicum* in China markedly declined in the 1990s as a result of a concerted effort of the Chinese government, backed by the 10-year World Bank loan project for schistosomiasis control (Chen et al., 2005). Recently, the re-emergence of schistosomiasis japonica was reported in the mountainous areas (Utzinger et al., 2005; Liang et al., 2006). We only found light infections, probably a result of repeated rounds of mass administration of praziquantel.

The achievements of the Chinese schistosomiasis control programme bring along new challenges. For example, the low infection intensity makes the Kato–Katz technique increasingly unreliable and the examination of a single stool sample can result in a considerable underestimation of the true prevalence (Yu et al., 1998; Zhu, 2005; Wang et al., 2006a). The increased sampling and diagnostic effort in three villages resulted in the detection of 21 additional *S. japonicum* infections. Interestingly, in one of these three villages, the Kato–Katz method performed on a single stool specimen failed to identify any of the four cases, and in the other two villages, the more intensive diagnostic approach detected additional cases. With the advent of sensitive serological tests for schistosomiasis japonica in the 1980s, it has become a common practice in China to first screen the population by ELISA, followed by stool examination using the Kato–Katz technique among seropositives. In our study, this would have left undetected 3 of the 38 cases in the “known” schistosome-

endemic villages (sensitivity: 92.1%). An additional 4 egg-positive cases were detected in villages previously thought to be *S. japonicum*-free. The standard tools for schistosomiasis control in China were developed in, and adapted to, the larger and ecologically more uniform endemic areas in eastern China. However, the focalized transmission in mountainous areas and the resulting small-scale classification into *Oncomelania*-infested, hence endemic communities, and non-endemic villages can result in residents of non-endemic villages being infected but not covered by control campaigns.

Higher education and socio-economic status were positive predictors for hand washing, which in turn was protective against *A. lumbricoides*, *T. trichiura* and cysticercosis seropositivity. Whilst 13.5% of the least poor families lacked sanitation facilities, the respective percentage in the ‘most poor’ families was 86.3%. The higher prevalence of *Taenia* spp. infection and cysticercosis-specific antibodies in poorer population strata and in mountainous areas could result from the joint effects of more precarious hygiene conditions, a higher prevalence of small-holder pig farming than in more affluent areas (94.9% versus 62.3%), and the absence of praziquantel-based mass chemotherapy in non-schistosome-endemic areas. Praziquantel is the drug of choice for the treatment of taeniasis and cysticercosis (Ito et al., 2003; Chen et al., 2004). Raw pork and raw beef was consumed by 90.0% and 25.9% of the participants, respectively. Whilst the consumption of raw pork was equally common in Han and Bai and across all socio-economic strata, Han consumed raw beef more frequently (49.4%) than Bai (20.1%). The prevalence of raw beef consumption steadily increased with socio-economic status, from 5.9% among the poorest to 45.3% among the richest. The almost universal consumption of raw pork prevents its conclusive association with any infection. Therefore, the observed association with trichinellosis might be a chance finding, but is supported by observations in other areas of China (Wang et al., 2006b).

We observed an inverse relationship between the mean socio-economic status of the families and distance from the main road. The highest proportions of very poor and most poor families were found in mountainous areas without road access. In our future work, we plan to carry out more detailed analyses of the relationship between the disparity in socio-economic conditions and the local epidemiology of parasitic diseases taking into account between and within village variation. This kind of information will clarify the observed relations and aid in the tailoring of setting-specific control approaches.

Recently, soil-transmitted helminth infections and other so-called neglected tropical diseases have attracted



new attention (Molyneux et al., 2005; Hotez et al., 2006; Utzinger and de Savigny, 2006). It has also been argued that integration of parasite-specific control programmes could use synergies and hence become more cost-effective (Brady et al., 2006; Engels and Savioli, 2006; Fenwick, 2006). Research, monitoring and control of schistosomiasis have a long history in China (Utzinger et al., 2005) but until now, the respective infrastructure has hardly been used for other public-health activities. The present study was carried out using staff and tools available in local schistosomiasis control institutions. As the prevalence of *S. japonicum* declines, there is scope to use this established control and surveillance infrastructure for other parasites, especially soil-transmitted and food-borne helminths.

## Acknowledgements

We thank the staff of the Institute of Research and Control of Schistosomiasis in Dali prefecture and the Eryuan county Schistosomiasis Control Station for their commitment in the current study. We are grateful to the local authorities for their support during the study, and the participants who provided multiple stool samples. This investigation received financial support from the Swiss National Science Foundation (project no. PPOOB-102883), the National Natural Science Foundation of China (no. 30590373), the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (no. A30298), the Freiwillige Akademische Gesellschaft, Basel and the Commission for Research Partnerships with Developing Countries (through the SDC-sponsored programme “Jeunes Chercheurs”). P. Steinmann is grateful to the Janggen-Pöhn Stiftung for a personal stipend for the final year of his Ph.D. thesis.

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