

A BASELINE STUDY ON THE IMPORTANCE OF BOVINES FOR HUMAN *SCHISTOSOMA JAPONICUM* INFECTION AROUND POYANG LAKE, CHINA

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Abstract. We hypothesize that bovine infections are responsible for the persistence of human schistosomiasis transmission in the Yangtze marshlands of China. To test this hypothesis, we are carrying out a comparative intervention among four administrative villages in the Poyang Lake region, Jiangxi Province, two of which are experimental and two are control. The primary design involves treating, at the onset of the study, all the inhabitants in all four villages with praziquantel and all the bovines in two villages (the experimental or intervention villages). Following treatment, rates of reinfection in people of all villages, and in bovines in the experimental villages, will be assessed as will the ongoing prevalence of infection in bovines in the control villages. Before treatment, the prevalence and intensity of infection among humans and bovines was ascertained in the four villages. Our study design and baseline information are presented here, along with a description of the ecology of the study villages.

INTRODUCTION

Currently, 0.9 million people are infected with the Oriental schistosome, *Schistosoma japonicum*, in areas situated primarily along the middle and upper reaches of the Yangtze River.¹ Within China, the Poyang Lake region represents one of the last strongholds for schistosomiasis in the marshland ecotype. According to the 1995 province-wide sampling survey, 1,125 villages were endemic with an underlying human prevalence ranging from 5% to 30%.² Among the hyperendemic communities, the bovine (cattle and water buffaloes) prevalence was approximately 13%. Although the infection rate of cattle was reported to be a little higher than that of water buffaloes, the latter (220,000 animals) are ten times more numerous. Moreover, the survey revealed that more than 90% of schistosome eggs in the flood plains were excreted by buffaloes. It is noteworthy that the average stool weight of cattle per day (estimated to be 25 kg) is 100 times that of humans (250 g). Buffaloes produce 50–60 kg of stool per day.

Given this evidence, we hypothesize that bovines, especially buffaloes, are responsible for the persistence of human schistosome transmission in the Yangtze marshland region where endemic schistosomiasis remains uncontrolled. Since 1992, praziquantel has been used repeatedly as chemotherapy following World Bank-Chinese Schistosomiasis Control Program guidelines, yet infection remains stubbornly above a prevalence of 5%.^{3,4} Control of snails is not possible due to the large areas of lakeside marshland involved and the inability to manipulate the environment as has occurred in other areas of China where control has been achieved.^{5–7} The chemotherapy program in the marshland areas is focused on human populations.⁸ Animal treatment is attempted but is much more difficult to accomplish for routine control. Although praziquantel works well on cattle and buffaloes they are difficult to reach for treatment, their water exposure remains substantial and unaltered, their worm burdens are often very high, and their population changes frequently due to a high birth rate (> 10% per year) and trading of animals from one area to another (~ 10% per year). Thus, routine

chemotherapy programs for bovines have achieved limited coverage (< 50%) and limited success. At present, chemotherapy is supplied biannually to both humans and bovines in areas with persisting moderate human prevalence of infection (5–15%) and less frequently in areas of lower prevalence.⁹

A National Institutes of Health-funded Tropical Medicine Research Centre (TMRC), based at the Institute of Parasitic Diseases in Shanghai, is developing and testing anti-schistosome vaccines for buffaloes.^{10,11} As part of the TMRC program, the project described here, was designed to measure and mathematically model the importance of buffaloes in transmission of schistosomiasis to humans. This information will be vital when it is time to field test buffalo vaccines and it will also connect with ongoing research on oncomelanid snail dynamics.

We chose four administrative villages for the study: two as experimental areas (intervention) and two as control areas (an administrative village may have two or more natural villages). The design involved treating, at the onset of the study, all the inhabitants in all four villages with praziquantel and all the buffaloes/cattle in two villages (the experimental villages). Before treatment, the prevalence of infection in humans, bovines, and snails was established. After treatment, rates of re-infection in people of all villages, and in bovines in the experimental villages are being assessed as well as the ongoing prevalence of infection in bovines in the control villages. If our hypothesis on the importance of bovines is correct, reductions of infection in bovines will reduce snail infection and substantially decrease human transmission. Here we present the 1998–1999 cross-sectional baseline epidemiologic data for the four study villages. A companion paper¹² describes the snail sampling procedures used to assess the ecology, density, and distribution of infected *Oncomelania* snails in the four villages using geographic information systems (GIS) and remote sensing technology. Subsequent articles will discuss the longitudinal data and mathematically model the modes of bovine transmission for three years after the baseline survey.

MATERIALS AND METHODS

Study areas. Four selected administrative village communities (introduced in Davis and others;¹³ see also Davis and others¹²) around Poyang Lake in Jiangxi Province were divided into two groups: two intervention (experimental) villages (bovines treated with praziquantel) and two control villages (bovines not treated). The intervention villages comprised Jishan Administrative Village in Yongxiu county, and Dahuang Administrative Village in Duchang county. The control areas comprised Xinhua Administrative Village in Xinzi county and Hexi Administrative Village in Yongxiu county. The GIS-facilitated maps of Poyang Lake showing the localities of the four villages are presented in Figures 13 and 14 in Davis and others.¹³

Dahuang Administrative Village, consisting of 12 natural villages, is situated in the northeastern sector of Poyang Lake. The total population at the commencement of the study was 2,299, with most working in either the agricultural or fishing industry. The main agricultural products are rice, sweet potatoes, peanuts, and beans. The villages are adjacent to the lakeshore and marshland and residents contact potentially endemic waters primarily through occupation or daily activity. In the dry season, bovines graze on the marshlands. Pigs are raised within pigsties throughout the year. Since 1992, regular control measures have been taken by the local schistosomiasis station. In 1997, the positive rate for schistosomiasis by indirect hemagglutination assay among residents was 30% (401 of 1,335). As a result, mass chemotherapy was carried out in three of the natural villages and selective treatment in the remainder. The density of positive snails was recorded in 1990 at 0.026/m². It is noteworthy that Dahuang is the smallest of the four administrative villages in the study and differs from the other three in that there are no buffalo here, only cattle (numbering 168 in total) grazing on 0.35 km² of land.

Jishan, consisting of eight natural villages, is located on Jishan island (5 km² in area) in the western sector of Poyang Lake. It is a typical heavy endemic area and had a total population of 2,017 at the commencement of the study. On the west side of the island, there are large areas of snail-infested marshland. Serious flooding occurred on the island in 1998 and 1999, which caused the evacuation of three whole villages. There are approximately 1,000 residents in the remaining four villages. Approximately 30% of the residents (mainly young people) work off the island for extended periods of time and thus only 600 villagers are exposed to infection by *S. japonicum*. Forty-six percent of the population is engaged in agricultural activities while the remaining individuals split their time between agricultural and fishing activities. There are 665 buffaloes on the island, but this number tends to fluctuate due to the buying, selling, death, and birth of individual animals. The entire grazing range is 12.1 km², the largest of the four study areas. According to epidemiologic data collected in 1996, 17% of the residents and 20% of the buffaloes were infected with *S. japonicum*, despite annual mass chemotherapy.

Xinhua, consisting of 19 natural villages, is situated in the northwestern sector of Poyang Lake. The total population is estimated to be 1,000 residents, with most engaged in either agriculture or fishing. All households raise pigs (~ 500 head

in pigsties, but buffalo (168) usually graze on the marshlands. Selective chemotherapy was administered to residents who were either stool-positive for eggs, or presenting with symptoms and signs associated with disease in 1995. In 1997, the prevalence among residents and bovines was 8% (42 of 500) and 18% (6 of 34), respectively. There are approximately 1.18 km² of grazing land and 267 × 10⁴ m² of snail-ridden area.

Hexi, consisting of six natural villages, is located on Hexilong Island in the western sector of Poyang Lake. There are 1,060 residents living on the island but one-third of them usually migrate for temporary work. Like the other study communities, the main daily activities of residents involves agriculture and fishing. One of the villages is situated only 20 meters from the marshland while the remaining five are 200–500 meters away. This island was also heavily hit by floods in 1998–1999, which forced the migration of many families from the island. The total grazing area (3.69 km²) ranks second among the four communities surveyed. There are 364 buffaloes on the island and all pigs are housed in pigsties.

Hexi and Jishan villages are similar in ecologic complexity and thus are well paired as control and experimental villages; the same holds true for the smaller villages (in area) of Dahuang and Xinhua.¹²

Study design. The field study commenced in late 1998 and will last three years. The overall study design is shown in Figure 1. All residents aged five to greater than 60 years of age in the four administrative villages were treated and cured at the commencement of the study. The at-risk population is being followed for three years (over six transmission periods) to measure exposure to water at snail-infection sites and to determine the incidence and intensity of re-infection. At the end of the first, second, and third years of the study, all those re-infected are to be treated. All bovines in the intervention areas were treated and cured of infection at the start of the study and 12 and 24 months later. By comparing human infection incidence and serial snail infection rate in the bovine-treated and bovine-untreated communities, the importance of infected buffaloes in disease transmission will be determined.

At baseline a medical questionnaire was administered to all study participants. The questionnaire included items such as ethnographic background, disease history, and history of water contact outside the dikes that surround the lake. This was followed by a stool survey on a random age-sex stratified sample of the at-risk population, defined as any persons reporting on the questionnaire any contact with water in Poyang Lake. This permitted us to efficiently estimate the pretreatment prevalence of infection in each study area. We collected stools from approximately 20 males and 20 females chosen at random from the at-risk list for each of 7 age groups in each village (5–9, 10–14, 15–19, 20–29, 30–39, 40–49, 50–60, and > 60 years). The samples (Table 1) numbered approximately 280 persons per village, except for Xinhua where we performed the stool survey on almost the entire population to complete a previous study of schistosomiasis in that area.

Each pretreatment stool examination included two 41 mg of Kato-Katz (KK) smears obtained from one stool specimen per person. The whole population in all study areas was then

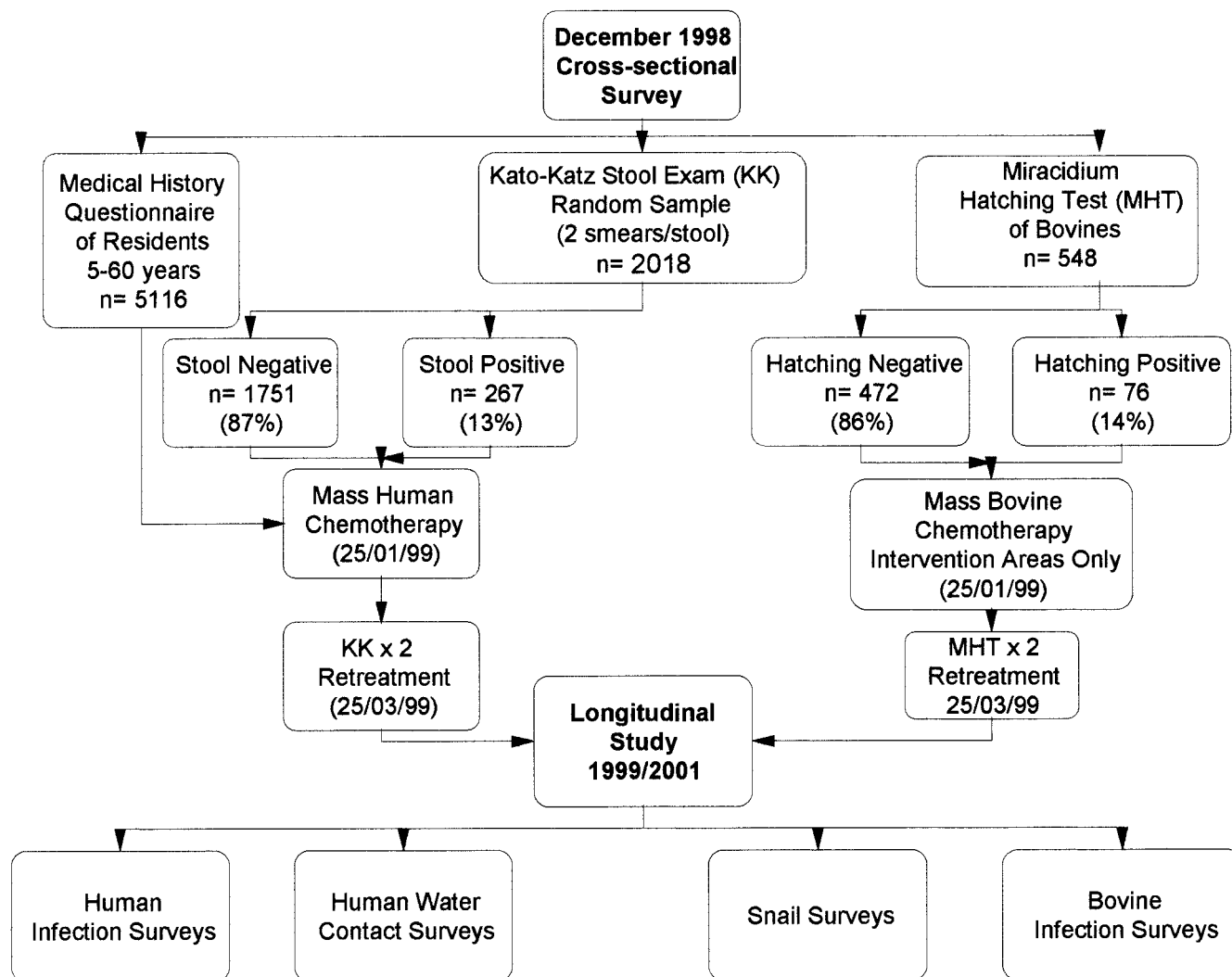


FIGURE 1. Flow diagram illustrating the procedures carried out in the 1998–1999 cross-sectional survey and their resulting epidemiologic outcomes. Also depicted are the additional studies planned for subsequent years.

treated with praziquantel (40 mg/kg) and one month later the subset known to be at risk of infection was rechecked again for persisting infection (one stool, 3 KK smears/person). If still infected (at least one positive smear), they were re-treated and checked again one month later (one stool, 3 KK smears). No individual required a third baseline treatment. We thus efficiently obtained estimates of baseline (pretreatment) infection prevalence (one stool, 2 smears, all age-sex

groups in a random sample of those at risk). Furthermore, we efficiently demonstrated that all people were uninfected after treatment at the start of our ongoing longitudinal estimate of re-infection (3–5 negative smears per person in a total of 2 stools with at least the last 3 smears all negative).

After completion of the stool surveys, human activity diaries are completed annually for the entire at-risk human population during the spring (May–June) and fall (October)

TABLE 1
Distribution of uninfected and infected individuals within study villages*

Village	Uninfected†		Infected (eggs/g)					
			12–100 epg (n = 185)		101–400 epg (n = 58)		>400 epg (n = 24)	
	0 epg n	(n = 1,751) %	n	%	n	%	n	%
Jishan	228	80	45	16	12	4	1	0.3
Dahuang	243	87	26	9	6	2	4	1
Xinhua	1,026	88	88	7	37	3	17	1
Hexi	254	89	26	9	3	1	2	0.7

* epg = egg per gram (of feces).

† Those egg-negative for *Schistosoma japonicum* by the Kato-Katz thick smear stool examination.

TABLE 2
 Characteristics of the combined human population according to age, sex, prevalence, and intensity of *Schistosoma japonicum* infection

Age group (years)	Males			Females			Total		
	n	% Infected	GM*	n	% Infected	GM*	n	% Infected	GM*
5–9	101	12	582	70	13	399	171	12	503
10–14	231	20	121	174	13	227	405	17	156
15–19	115	19	75	69	13	145	184	17	96
20–29	112	17	153	118	12	107	230	14	134
30–39	136	13	33	186	11	192	322	12	118
40–49	167	15	79	198	7	67	365	10	75
50–60	168	12	202	160	9	32	328	10	132
>60	10	20	54	3	0	0	13	15	54
Total	1,040	16	145	978	11	165	2,018	13	153

* GM = geometric mean egg counts/gram of stool for infected individuals.

transmission periods. Approximately 200 residents from each village are completing the activity diaries. Local doctors and teachers are visiting each study participant and writing the records twice a week. The activity diary questions include the location, frequency, duration, time of day, and body part immersed for each contact with water.

A list of the age, sex, identification, and owner of each bovine in the four administrative villages under study was also obtained. From the list, a random sample of males (15) and females (15) in each of the following three age groups was obtained: < 1 year, 1–3 years, and > 3 years. This gave a total of ~ 90 buffaloes per village, except for Dahuang, where nearly all of the cattle (152 of 182) were tested. For each bovine included in the sample, a fresh stool specimen (~ 200 g) was obtained and a 50–60 g sub-sample was taken for a miracidial hatching test (MHT). Hatching was done at optimal temperature (24–28°C), achieved by pre-heating the room where the MHT was to be performed and monitoring it by thermometer. Any bovines identified as positive by MHT had 50 g of the unused portion of their original stool sample subjected to an egg count procedure, with the stool suspended in the water and passed through sieves to remove fiber (mesh sizes of 30 and 150 per inch) and then suspended in a nylon bag that captures the eggs in the sediment, which on resuspension was examined and counted by a standard method to calculate number of the eggs per gram (epg) (of feces) for all positive buffaloes.

The snail survey sampling methods have been given in detail in Davis and others.¹² Briefly, using topographic maps, a series of 10,000 m² areas (called squares) was selected from representative areas of the grazing range of each village. Twenty 100 m² units (areas) were chosen at random from the 100 such numbered areas in each square. All snails were collected from a 4 m² frame placed in the center of each unit. The snails were scored for living and dead; all living snails are crushed to determine the number infected with *S. japonicum* and with non-*japonicum* trematodes. A GPS (geographic positioning system) record was made to provide exact coordinates for each frame. The GPS data are used in conjunction with remote sensing satellite images to provide precise mapping of localities. Collections are made twice a year, in the spring before the annual flood and in the fall after the floods have subsided. The percentage of grazing land with and without snails is then calculated. The data permit assessing the distribution of infected snails relative to bovine and human behavior through time.

Ethical approval. Written ethical approval for this study was obtained from the National Institutes of Health (Bethesda, MD) and at the national, provincial, and district level within the People's Republic of China. Oral informed consent was obtained from all adult subjects and from the parents or guardians of minors. Study participants identified as stool egg-positive for schistosomiasis were treated with 40 mg/kg of praziquantel, which is the current dosage recommended by the World Health Organization. One month after the initial treatment all treated subjects and bovines (20 mg/kg) were re-examined and retreated if positive.

Statistical analysis. SPSS version 9.0 for Windows (SPSS, Inc., Chicago, IL) was used for further statistical processing. The intensity of infection of infected groups was expressed as geometric mean egg counts per gram of stool. For whole populations the geometric means were calculated using a log (n + 1) transformation. The chi-square test was used to evaluate differences between relative frequencies. The Student's *t*-test or analysis of variance were used to detect differences between group means. The minimum level considered for statistical significance was set at $P < 0.05$.

RESULTS

Population descriptors. A medical questionnaire was administered to 5,116 (Dahuang = 1,170, Hexi = 1,063, Jishan = 778, and Xinhua = 2,105) individuals (52% male, mean age = 29 years) in their homes. Forty percent of the sample reported at least a primary level of education with most (44%) engaged in fishing. Seventy-five percent of the study participants stated that they had been previously diagnosed with schistosomiasis, but only 2% ever had advanced clinical features defined as dwarfism, ascites, splenomegaly, or hematemesis. The subjects were treated an average of three times in the past, with 47% having been treated in the previous two years. Other symptoms commonly reported over the previous two weeks were diarrhea (15%), general weakness (10%), and fever (7%). Fifty-four percent of the population reported weekly water contact and 65% stated frequent flood water contact during the 1998 storms.

Human prevalence and intensity of infection. Table 2 shows the prevalence and intensity of infection of the human population. In general, 13% were infected, with a geometric mean (GM) intensity of 153 epg. The highest prevalence and intensities were evident in the younger (< 15 years old) age groups. There were statistically significant ($P < 0.001$) dif-

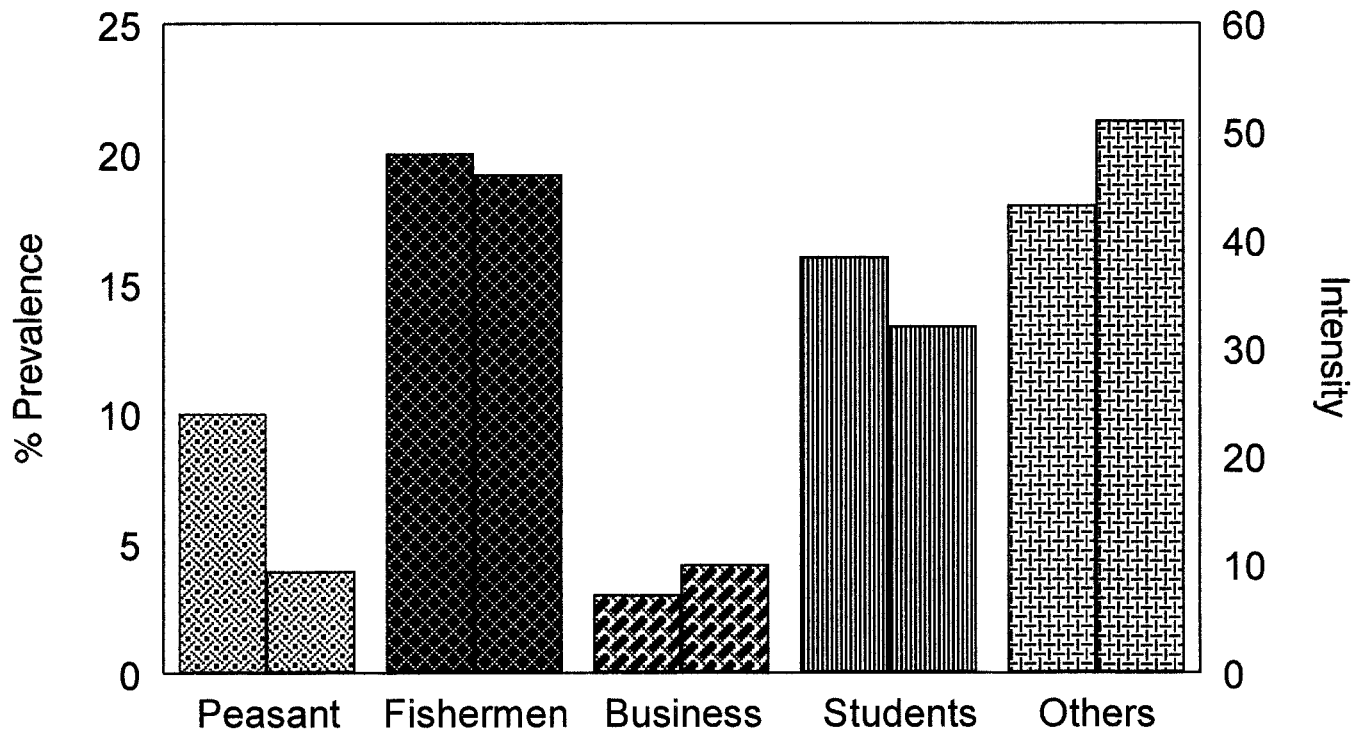


FIGURE 2. Prevalence (first column) and intensity (geometric mean log egg intensity/gm) (second column) of *Schistosoma japonicum* grouped by occupation in the four surveyed administrative villages.

ferences between the sexes for prevalence but not intensity. Males were more likely to be infected (16% versus 11%) but their infections were not as severe (145 egg versus 165 egg). When the four study communities were classified according to the percent uninfected, lightly infected (12–100 egg), moderately infected (101–400 egg), and heavily infected (> 400 egg) with *S. japonicum*, the distribution patterns for uninfected and infected individuals were similar. Most of the tested study population were uninfected (80% in Jishan, 87% in Dahuang, 88% in Xinhua, and 89% in Hexi). Lightly infected individuals were the next largest group (16% in Jishan, 9% in Dahuang, 7% in Xinhua, and 9% in Hexi), followed by moderately infected individuals (4% in Jishan, 2% in Dahuang, 3% in Xinhua, and 1% in Hexi). Only a small percentage were heavily infected (0.3% in Jishan, 1% in Dahuang, 1% in Xinhua, and 0.7% in Hexi). The intensity profiles of the respective villages varied significantly ($P < 0.027$) from each other (Table 1).

Human exposure. Although 54% of the study population reported weekly water contact, only 24% of the residents of Xinhua reported such contact. This outcome was statistically different ($P < 0.0001$) from that reported for Hexi (70%), Jishan (70%), and Dahuang (74%). Among occupational groups (Figure 2), fishermen have the highest prevalence and intensity of infection among the communities. Those categorized as “other” were also heavily infected and thus this group needs to be investigated further. Mode (activity) of water contact was also found to vary significantly ($P < 0.0001$) among the study communities. Figure 3 shows that fishing is the primary activity on the two island administrative villages (Hexi and Jishan), while collecting firewood

near pools of water was the common mode of exposure in Xinhua and Dahuang.

Bovine prevalence and intensity of infection. Table 3 shows the prevalence and intensity of the sampled bovine population. In general, 14% of the tested bovines were infected with a GM intensity of 42 egg. The highest prevalence (27%) was found among bovines 6–12 months old, while the highest GM intensity of infection (69 egg) was found among bovines 12–24 months old. There was no statistical difference between the sexes for either prevalence or intensity of infection. However, most (55%) of the infected bovines tested were found in Dahuang village, and this is where all the cattle reside.

DISCUSSION

Schistosomiasis in the Yangtze River basin remains one of the last strongholds for this disease in China despite 40 years of concerted control efforts by the government. Chemotherapy has been the cornerstone of most control efforts to date. With the end of the World Bank Loan Schistosomiasis Control Program in 2000 and the completion of the Three Gorges Dam by 2009 it is feared that, with limited financial resources, schistosomiasis will once again become a serious problem in lake and marshland localities. In the Poyang Lake region, mass chemotherapy has been used extensively over the past decade,^{3,4,14} but the underlying prevalence of human schistosomiasis in the region remains stubbornly well above 5%. A large number of infected bovines and continuing transmission suggest that bovines are a major potential reservoir for schistosomiasis transmission and a

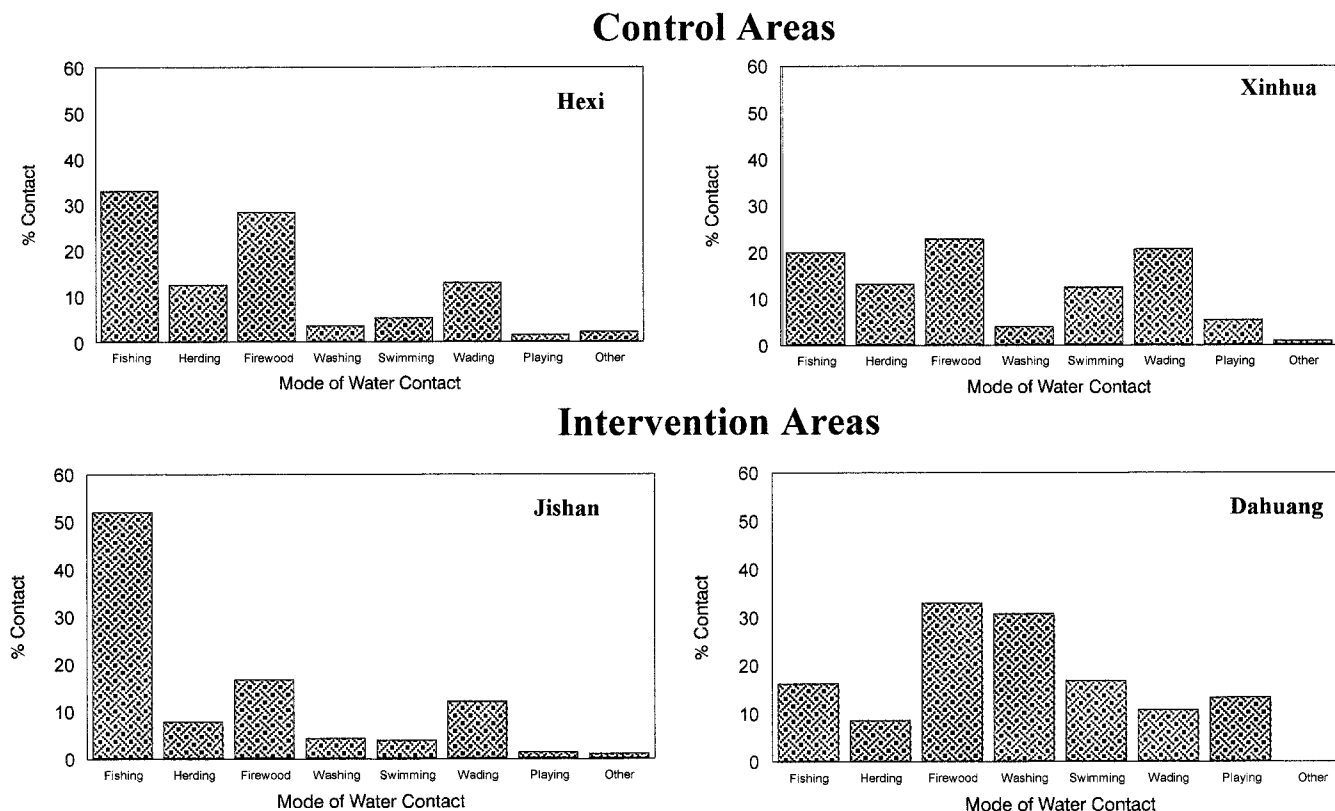


FIGURE 3. Reported human water contact for various activities within each of the respective study areas.

threat to eradication programs. To ascertain their actual contribution in the transmission of human schistosomiasis, we initiated a comparative intervention that will help to determine the bovine contribution to human infection.

We have described our study design and baseline data for future reference as the impact of the intervention unfolds. In a subsequent paper, we will report the rates of re-infection in people of all villages, and in the bovines in the intervention villages as well as the serial prevalence estimates for infection of bovines in the control villages. We will then be able to assess the importance of buffaloes and cattle for maintaining transmission of *S. japonicum* in this lake region of China that has been resistant to control for many decades.

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TABLE 3

Characteristics of the combined bovine population according to age, sex, hatching, and intensity of *Schistosoma japonicum* infection

Age group (months)	Males			Females			Total		
	n	% Hatching*	GM†	n	% Hatching*	GM†	n	% Hatching*	GM†
0-6	32	13	37	43	5	41	75	8	39
6-12	24	38	42	25	16	38	49	27	41
12-24	40	3	46	40	10	75	80	6	69
24-36	14	67	18	42	19	47	56	16	44
36-48	13	15	3	33	12	60	46	13	41
48-60	14	21	27	30	17	37	44	18	33
60-72	8	0	0	31	19	28	39	15	27
72-84	8	25	23	20	10	13	28	14	17
84-96	15	13	40	43	19	50	58	17	47
>96	17	12	113	56	13	35	73	12	52
Total	185	14	40	363	14	43	548	14	42

* % Hatching = percent miracidia hatching from stool.

† GM = geometric mean egg counts/gram of stool for infected bovines.

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