EPIDEMIOLOGY OF HUMAN INTESTINAL NEMATODE INFECTIONS IN WUJIANG AND PIZHOU COUNTIES, JIANGSU PROVINCE, CHINA

Sun Fenghua[1], Wu Zhongxing[1], Qian Yixing[1], Cao Hangqun[1], Xue Haichou[2], Ren Hainan[3], Xiao Shuhua[2], Zhan Bin[2], John M Hawdon[3], Feng Zheng[2], and Peter J Hotez[3]

[1]Jiangsu Institute of Parasitic Diseases, Wuxi 214064, PR China; [2]Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai 200025, PR China; [3]Departments of Epidemiology and Public Health and Pediatrics, Yale University School of Medicine, New Haven, CT 06520, USA

Abstract. Intestinal nematode infections are considered highly endemic in the Chinese province of Jiangsu. In May 1997, the prevalence of intestinal nematodes infections was determined among all of the inhabitants aged 5 to 65 of the southern Jiangsu village of Yaojiakon (Wujiang County) and the northern Jiangsu village of Jianmiao (Pizhou County). It was determined that the prevalence of ascariasis, trichuriasis and hook worm infection was greatly reduced compared to when it was last measured in 1990. The reduction was noted to be particularly impressive in Yaojiakon village where the prevalence of ascariasis and trichiuriasis fell to 2% and 1.5% respectively. Much of this reduction was attributed to improvements in economic development which have occurred in southern Jiangsu Province at a rapid pace. In contrast, the reduction in nematode infections among villagers living in northern Jiangsu was more modest. The most striking reduction in both villages was in school-aged children who since 1988 have received yearly treatments with anthelminthic drugs. The intensity of nematode infections was investigated for hookworm where 70% of hookworm-infected Yaojiakon villagers were found to harbor light infections (< 400 eggs per gram) compared to 83% of hookworm-infected Jianmiao villagers. *Necator americanus* was the predominant hookworm in Yaojiakon village (South), whereas *Ancylostoma duodenale* predominated in Jianmiao village (North). The majority (76%) of hookworm-infected patients developed IgG antibodies against *N. americanus* antigen, although 20% of uninfected patients living in the village also had circulating antibodies. Intestinal nematode infections continue to be a significant public health problem in Jiangsu Province although their prevalence has decreased since 1990.

INTRODUCTION

Intestinal nematode infections caused by *Ascaris, Trichuris* and hookworms are highly endemic to China, with the numbers of cases based on a nationwide survey conducted between 1988 and 1992 (comprising 1,477,742 fecal examinations) estimated to be 531 million, 212 million and 194 million, respectively (Yu *et al,* 1994; Hotez *et al,* 1997). The Chinese nationwide survey determined that Jiangsu Province, like many Yangtze River provinces was highly endemic for intestinal nematodes. Based on 63,699 fecal examinations the prevalence of *Ascaris, Trichuris* and hookworm infection was entimated to be 39.5%, 27.3% and 31.8%, respectively (Xu *et al,* 1995). However, the last 5 years since the completion of the survey have witnessed two important changes in Jiangsu Province. First, economic development has occurred at a rapid pace, probably more so than any other Yangtze River province. Second, the Chinese Ministry of Health has aggressively sought to diminish the prevalence and intensity of intestinal helminth infections through widespread treatment with anthelminthic chemotherapeutic agents. Mass chemotherapy has also been implemented in association with health education. During the nationwide survey completed in 1992, the villages of Yaojiakon in Wujiang County and Jianmiao in Pizhou County were estimated to have intestinal nematode prevalences of 54.7% and 76.9%, respectively. These two villages were revisited in 1997 to determine the prevalence and intensity of hookworm, as well as ascariasis and trichuriasis.

Correspondence : Wu Zhongxing, Jiangsu Institute of Parasitic Diseases, Wuxi 214064, PR China.

MATERIALS AND METHODS

Sample selection and fecal examination

Fecal examinations were performed on all local residents of Yiaojiakon village (Wujiang County)

and Jianmiao village (Pizhou County) between the ages of 5 and 65. Human investigations were conducted under a single project assurance from the office of research protection of the National Institutes of Health (Bethesda, MD, USA). Samples were collected in May of 1997. Identification of intestinal nematode eggs (*Ascaris, Trichuris* or hookworm) was determined by brine flotation. Quantitative hookworm egg counts were determined in patients whose fecal examinations were positive for hookworm using the Kato-Katz method (Suzuki, 1980; Katz *et al,* 1972). Hookworm species (*Ancylostoma duodenale* or *Necator americanus*) was determined by morphological identification of third-stage infective larvae which were reared from eggs (Sasa *et al,* 1974; Wu *et al,* 1965). From each patient, at least 100 larvae were identified for mixed infections, while 200 larvae were identified to determine if a patient had infection with a single species of hookworm.

Anthelminthic treatment and adult worm recovery

At each of the two sites 30 village residents with diagnosed hookworm infection were treated orally with pyrantel pamoate at a daily dose of 10 mg/kg (maximum 1.5 g containing 0.5 g of free base) in two equally divided doses spaced approximately 8 hours' apart. Following anthelminthic treatment, all feces from each patient were collected for 48 hours, and washed through sieves with water. Two different sized sieves (1 mm and 0.3 mm) were

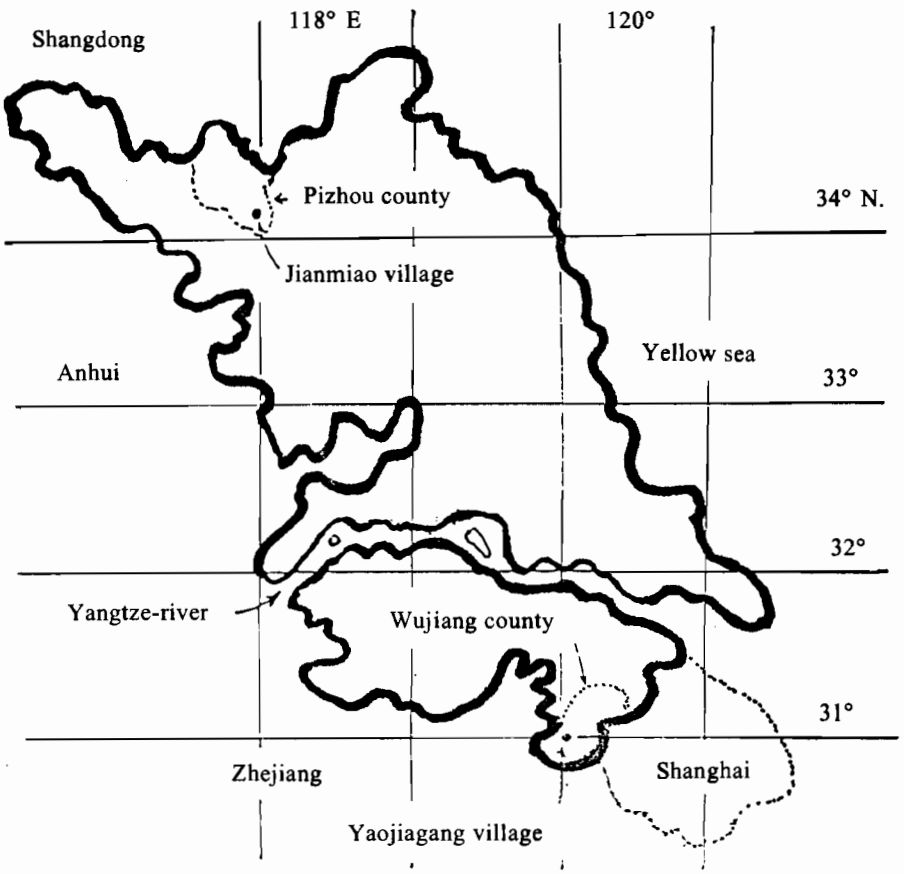

Fig 1–Map of geographical distribution of hookworm infection in Wujiang and Pizhou County, Jiansu Province.

used. The adult hookworms were individually picked by careful examination of the sediment, and worm number was recorded.

Hookworm serology

Serum samples were collected from 55 hookworm-positive and 20 hookworm-negative residents from each village. As negative controls, 17 serum samples were collected from matriculating medical students of Shanghai Second Medical University at the time of their entrance physical examinations. Anti-hookworm antibodies were measured in sera diluted 1:100 with 5% nonfat dry milk in phosphate buffered saline (PBS) containing 0.3% tween 20, by enzyme-linked immunosorbent assay (ELISA). Horseradish peroxidase-conjugated goat antihuman IgG (Biorad) was used as the second antibody (Xue *et al,* 1992a) at a 1:10,000 dilution. Soluble hookworm antigen was prepared from *Necator americanus* adult hookworms (SNaA) using a previously described method (Xue *et al,* 1992b).

RESULTS

General characteristics of the study sites

Yaojiakon village (Wujian County) is located in southern Jiangsu Province (latitude. 31°18 ; longitude 120°31 ; elevation. 2 meters) (Fig 1) The annual average temperature and relative moisture are 15°C and 78-82% respectively. The major agricultural products are rice,wheat, mulberry and rape - both chemicals and human feces are used for fertilizer. Currently fewer than 50% of the residents are engaged in agricultural pursuits.

Jianmiao village (Pizhou County) is located in the northern part of Huanghuai Plain, Jiangsu Province (latitude. 34°21 ; longitude.118°02 ; elevation 20 meters) with annual average temperature of 13.5-14.5°C and relative moisture of 78-82%. The major agricultural products and fertilizer used are similar to Yaojiakon village. It is estimated that approximately 80% of the residents are employed in agriculture.

Prevalence of nematode infections

Since 1990 the overall prevalence of intestinal nematode infections has dropped substantially in the two villages studied. In 1990 the prevalence of intestinal nematodes was estimated to be 54.7% in Yaojiakon (Wujian) and 76.9% in Jianmiao (Pizhou) villages. As shown in Table 1, these prevalence rates have decreased to 15.6% and 52.7%, respectively. The prevalence of each major intestinal nematode infection has decreased - the prevalence of ascariasis in Yaojiakon and Jianmiao decreased from 14.8% and 68.2% in 1990 to 2.0% and 28.9%, respectively; for trichuriasis the prevalence decreased from 21.6% and 36.4% in 1990 to 1.5% and 26.3%, respectively; and for hookworm infection the prevalence decreased from 36.9% and 42.4% in 1990 to 12.4% and 12.7%, respectively. Both in 1990 and in this study conducted in 1997, the prevalence rate of all three intestinal nematodes was much higher in Jianmiao village in the northern Jiangsu compared to Yaojiakon in southern Jiangsu. The dramatic decrease in ascariasis and trichuriasis which was noted to occur in Yaojiakon over the last five years accounted for much of this disparity. Hookworm infection prevalence rates are now about equal between the two villages.

The relationship between age and prevalence of the three intestinal nematodes infections is pre-

Table 1

The prevalance rate of helminths in two pilot sites.

Pilot site	No examined	Positive with helminths		Hookworm		*Ascaris*		*Ttichuris*	
		No.	%	No.	%	No.	%	No.	%
Yaojiakon	405	61	15.6	50	12.4	8	1.9	6	1.5
Jianmiao	475	248	52.7	60	12.7	136	28.9	124	26.3
Total	876	309	35.3	110	12.5	144	16.4	130	14.8

Table 2

The relationship between the prevalence of three helminths and the ages of residents in pilot site of Yaojiakon and Jianmiao.

Age (year)	No. examined	Positive		Hookworm		Ascaris		Trichuris	
		No.	%	No.	%	No.	%	No.	%
5-	37	0	0	0	0	0	0	0	0
11-	184	43	23.4	6	3.3	24	13.0	21	11.4
21-	133	64	48.1	15	11.3	33	24.8	28	21.1
31-	143	55	38.5	14	9.8	30	21.0	23	16.1
41-	181	67	37.0	32	17.7	26	14.4	30	16.6
51-	111	51	45.9	26	23.4	19	17.1	16	14.4
61-	87	29	33.3	17	19.5	12	13.8	12	13.8
Total	876	309	35.3	110	12.6	144	16.4	130	14.8

Table 3

Determination of egg number in each gram of feces (EPG) in 110 residents with hookworm infection.

Pilot site	EPG					
	<400	%	400-3,000	%	>3,000	%
Yaojiakon	35	70.0	12	24.0	3	6.0
Jianmiao	50	83.3	10	16.7	0	0
Total	85	77.2	22	20.0	3	2.72

Table 4

Species identification in third-stage infective larvae (L_3) and adult worms from 60 residents with hookworm infection .

Pilot sites	No . examined	L_3			Adult worm		
		Ad	Na	Mix	Ad	Na	Mix
Yaojiakon	30	0	25	5	0	27	3
Jianmiao	30	9	2	19	12	2	16

sented in Table 2. Of interest was the absence of infection among school-aged children ages 5-10. Since 1988 school-aged children in both villages have received once yearly combined anthelminthic drug treatment with mebendazole (100 mg) and levamisole (25 mg), beginning in kindergarten and continuing for the next 5-10 years. After age 10, the prevalence of ascariasis and trichuriasis was maximal among young adults aged 21 to 30, with a modest decrease thereafter. The prevalence of hookworm infection was the greatest among older adults.

Hookworm intensity and hookworm species

As shown in Table 3, on the basis of quantitative egg counts (< 400 epgs) the majority of patients harbored light hookworm infections. In Yaojiakon village 30% of those tested harbored moderate or heavy infections, while 16.7% harbored moderate infections in Jianmiao village.

As shown in Table 4, *N. americanus* was the predominant hookworm identified in the southern village of Yaojiakon village. No cases of pure *A. duodenale* infection were observed. In contrast, *A. duodenale* was the predominant hookworm found in the northern village of Jianmiao, although a few pure *Necator* infections were noted in addition to mixed infections. In Yaojiakon village the average *N. americanus* and *A. duodenale* adult worm numbers collected from 30 treated infected residents were 2.8 ± 3.1 (range of 1 to 15) and 0.1 ± 0.4 (range of 0 to 2), respectively. Three residents with mixed infection showed the average worm number of 10.0 ± 6.6 (range of 3 to 16). In Jianmiao village the average *N. americanus* and *A. duodenale* adult worm numbers collected from 30 treated residents were 0.9 ± 1.3 (range of 0 to 5) and 1.8 ± 1.3 (range of 2 to 7). The average worm number of 12 residents with mixed infection was 3.5 ± 1.6 (range of 2 to 7).

Hookworm serology

Hookworm IgG antibodies were measured by ELISA in 55 patients with hookworm infection (Table 5). The majority of patients (76.3%) recognized adult *N. americanus* antigen, although 20% of the village patients were negative for hookworm by fecal examination also had anti-adult *N. americanus* antibodies. All seventeen sera from Shanghai students were seronegative. Fewer hookworm-infected patients (32.7%) had antibodies to third-stage infective larval antigen prepared from *Ancylostoma caninum* (data not shown).

DISCUSSION

These studies demonstrate a significant decrease in the prevalence of intestinal nematode infections among villagers from two counties in Jiangsu Province, compared to when it was last measured in 1990. The most dramatic reduction occurred in the village of Yaojiakon (Wujiang County). This village is in the now prosperous southern region of Jiangsu Province, close to Shanghai. Economic development has occurred at a rapid pace in this village, with large numbers of young adults between the ages of 20-40 leaving agricultural pursuits in favor of commercial enterprises. In contrast, only a modest decrease in intestinal nematode prevalence was observed in Jianmiao village. Jianmiao is located in the northern county of Pizhou which has benefitted less from economic develop-

Table 5

Results of sera examined by enzyme-linked immunosorbent assay (ELISA), using soluble adult *Necator americanus* protein (SNaA) as antigen.

Serum	No. of cases	% SNaA	Antibody positive
Residents with egg-positive	55	42	76.3
Residents with egg-negative	20	4	20.0
Students from entrance physical examination	17	0	0

ment of the last seven years, and a majority of its inhabitants are still engaged in agricultural pursuits. In both villages, however, the prevalence of intestinal nematode infections fell to zero among school-aged children. This observation is particularly striking since throughout much of China school-aged children traditionally have had the highest prevalence of ascariasis and trichuriasis. In addition to economic development much of this reduction is attributed to an aggressive campaign to de-worm children once yearly with a benzimidazole anthelminthic. Typically, the child is treated when he first enters the school system at kindergarten, and then once yearly thereafter.

Infection intensity was investigated only for hookworm infection, where it was determined that the majority of patients harbored only light infections. The aggregative distribution of hookworm infection in which only one-third or fewer of the population harbors moderate or heavy infections has been observed previously throughout China as part of the nationwide survey. *N. americanus* infection was the predominant hookworm in the southern village of Yaojiakon whereas *A. duodenale* infection predominated in the northern village of Jianmiao. It has been noted *A. duodenale* infection is more common relative to *N. americanus* infection at more northerly latitudes in China (Xu *et al*, 1995). This observation may reflect the ability of *A. duodenale* to undergo arrested development in human tissues and thereby avoid exposure to colder and more unfavorable conditions at these latitudes. A high percentage of hookworm-infected patients developed anti-hookworm antibodies, although no effort was made to determine whether they were specific for hookworm (or any one hookworm species) or could possibly cross-react to other nematodes. Absence of specificity may account for the detection of anti-hookworm IgG antibodies in patients with negative fecal examinations. Alternatively, the presence of anti-hookworm IgG antibodies may reflect previous exposure. Recombinant hookworm antigens from Chinese human *A. duodenale* and *N. americanus* isolates are being generated in order to develop specific immunodiagnostic reagents.

ACKNOWLEDGEMENTS

The studies were supported by a Tropical Medicine Research Center (TMRC) award from the National Institute of Allergy and Infectious Diseases (NIAID) of the NIH (1 P50 A139461). Dr Hotez is an Established Investigator of the American Heart Association and was further supported by a Clinical Research Grant from the March of Dimes and NIH A132726.

REFERENCES

Hotez PJ, Feng Z, Xu LQ, *et al.* Emerging and reemerging helminthiases and the public health of China. *Emerg Infect Dis* 1997; 3:303-10.

Katz N, *et al.* A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 1972; 14: 397-400.

Sasa M, *et al.* Application of test-tube cultivation on method on the survey of hookworm and related human nematodes infection. *Jpn J Exp Med* 1974; 28: 129-37.

Suzuki N. Diagnostic method in intestinal helminth infection collected papers on the control of soil-transmitted helminthiasis. Asian Parasite Control Organization, 1980; 1:26.

Wu ZX *et al.* Studies on the morphological differentiation of the infective larvae of *Ancylostoma duodenale* and *Necator americanus*. *Acta Parasitol Sin* 1965; 2: 280-90.

Xu LQ, Yu SH, Jiang ZX, *et al.* Soil-transmitted helminthiases: nationwide survey in China. *Bull WHO* 1995; 73: 507-13.

Xue HC, He YX, Qiu LZ, Zhang YH. Studies on the strain difference of *Schistosoma japonicum* in the mainland of China. VIII. Immunoreactions in experimental animals. *Chin J Parasitol Parasit Dis* 1992a; 10: 104-7.

Xue HC, He YX, Zhu CW, Zhang YH, Qiu LS. studies on the strain differences of *Schistosoma japonicum* in the mainland of China. X. Comparison of SDS-PAGE protein patterns. *Chin J Parasitol Parasit Dis* 1992b; 10:0201-3.

Yu SH, Xu LQ, Jiang ZX, *et al.* Special report, nationwide survey of human parasites in China. *Southeast Asian J Trop Med Public Health* 1994; 25:4-10.