EPIDEMIOLOGY OF HUMAN ANCYLOSTOMIASIS AMONG RURAL VILLAGERS IN NANLIN COUNTY (ZHONGZHOU VILLAGE), ANHUI PROVINCE, CHINA: AGE-ASSOCIATED PREVALENCE, INTENSITY AND HOOKWORM SPECIES IDENTIFICATION

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Abstract. Hookworm infection has traditionally been highly endemic to Anhui Province, China. Zhongzhou village in southwestern Anhui was identified as an endemic focus of infection caused by the hookworm *Ancylostoma duodenale*. This species was found to predominate over *Necator americanus* in ratios ranging from 35:1 based on the recovery of third-stage infective larvae (L_3) to 21:1 based on the recovery of adult hookworms after anthelmintic chemotherapy. The overall prevalence of *Ancylostoma* infection in Zhongzhou was 33.2% with a greater prevalence among males. Unlike the age-associated prevalence patterns for *N. americanus* in Hainan and other southern Chinese provinces that show increasing prevalence with age and the highest prevalence among the elderly, the age-associated prevalence for *A. duodenale* in Zhongzhou exhibited a peak in middle-aged adults with subsequent decline. The age-associated intensity pattern exhibited a similar trend although the most of the hookworm infections were light or moderate infections as defined by quantitative egg counts.

INTRODUCTION

Intestinal nematode infections caused by the "unholy trinity" of *Ascaris lumbricoides, Trichuris trichiura* and the hookworms are major public health problems in China. Based on 1,477,742 fecal examinations conducted by the Ministry of Health between 1988 and 1992 it was estimated that 531 million, 212 million and 194 million Chinese were infected with these intestinal nematodes, respectively (Hotez *et al*, 1997). We have returned to some of these areas over the last two years to determine whether the economic improvements which have occurred in China since then have been associated with a corresponding decrease in the prevalence and intensity of these intestinal nematode infections. Indeed this appears to be true of Jiangsu Province near Shanghai where we observed reductions in the prevalences of ascariasis, trichuriasis and hookworm that were linked to improvements in the general standard of living (Sun *et al*, 1999). However, in other areas such as in Hainan and Sichuan, the prevalence and intensity of hookworm and other intestinal nematode infections have remained the same or have even increased (Chen *et al*, 1999; Liu et *al*, 1999). In these areas *Necator americanus* was noted to be the predominant hookworm.

Like Jiangsu and Sichuan, Anhui comprises one of the Chang Jiang (Yangtze River) valley provinces and was noted previously to have high rates of intestinal nematode infections (Xu *et al*, 1995). Based on 54,392 fecal examinations conducted between 1988 and 1993 in Anhui Province, it was noted that 46.4% of the populations harbor *Ascaris*, 17.4% harbor *Trichuris*, and 33.4% harbor hookworm (Hotez *et al*, 1997). Because *Ancylostoma duodenale* was noted to be an important hookworm in this region (Xu *et al*, 1995), we compared the prevalence and intensity of endemic ancylostomiasis in Anhui with our other study sites where *N. americanus* predominated. The present study reports on a focus of endemic hookworm where *A. duodenale* is almost the exclusive hookworm.

MATERIALS AND METHODS

Sample selection and fecal examinations

Fecal examinations were performed on 488 local residents of Zhongzhou village (Nanlin County). All of the residents selected were between 2 and

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65 years old. Non-written informed consent was obtained. The samples were collected from April to May of 1998. Identification of intestinal nematode eggs (*Ascaris, Trichuris*, or hookworm) was obtained by brine flotation. Quantitative hookworm egg counts were determined in 123 residents whose fecal specimens were positive for hookworm using the Kato-Katz method (Katz *et al*, 1972) and expressed as eggs per gram of feces (EPG). Hookworm infections were designated as light (1-399 EPG), moderate (400-3,000 EPG) or heavy > 3,000 EPG. Hookworm species (*Ancylostoma duodenale* or *Necator americanus*) was determined by morphological identification of third-stage larvae (L_3) which were successfully reared from eggs by coproculture from the feces of 84 residents (Wu *et al*, 1965; Sun *et al*, 1999). For identification of subjects with mixed or single hookworm infections, at least 100 L_3 were examined (Sun *et al*, 1999).

Anthelmintic treatment and adult worm recovery

In Zhongzhou village, 123 residents with diagnosed hookworm infections (often mixed with *Ascaris* and *Trichuris* infections) were treated with anthelmintics. The adult residents were treated with quantrel (Pfizer, Australia) each dose containing 100 mg of free base of pyrantel and oxantel,) at a dose of 10 mg/kg pyrantel and 10 mg/kg oxantel, for two consecutive doses separated over 8 hours. For children the dose was increased to 12 mg/kg using the same schedule. All feces from each treated subject were collected for 48 hours after the first administration as described previously (Sun *et al*, 1999). Two different sized sieves (1 mm and 0.3 mm) were used for washing and filtering the feces. The adult hookworms in the sediment were examined and individually picked for species identification and quantitation.

RESULTS

General characteristics of the study sites

Zhongzhou village (Nanlin County) is located in southwestern Anhui Province (lat 30° 55'; long 118° 17'), close to the Yangtze River (Fig 1). The climate is considered continental to sub-tropical with an annual temperature of 15.8 and rainfall of 130-140 cm. Most residents of the village are engaged in agricultural pursuits for the cultivation of cotton, peanuts, wheat, tobacco and vegetables. Human feces are still used as an important source of crop fertilizer in this region, although chemical fertilizers are

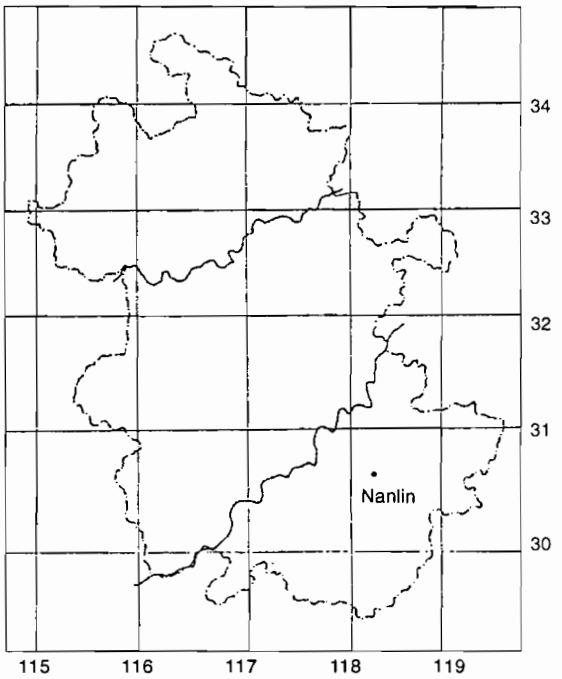

Fig 1–Map of geographical distribution of hookworm infection in Nanlin County, Anhui Province.

Table 1
The prevalence rate of helminth in Zhongzhou village.

Sex	No. examined	Positive with							
		Helminth		Hookworm		*Ascaris*		*Trichuris*	
		No.	%	No.	%	No.	%	No.	%
Male	282	154	54.6	107	37.9	7	23.8	23	8.2
Female	206	110	53.4	55	26.7	55	26.7	21	10.2
Total	488	264	54.1	162	33.2	122	25.0	44	9.0

Table 2
The relationship between the prevalence of three helminths and the ages of residents in pilot site of Zhongzhou village.

Age (year)	No. examined	Positive with					
		Hookworm		*Ascaris*		*Trichuris*	
		No.	%	No.	%	No.	%
2-	66	8	12.1	24	36.4	6	9.1
11-	77	7	9.1	20	26.0	17	22.1
21-	43	12	27.9	11	25.6	5	11.6
31-	68	30	44.1	18	26.5	3	4.4
41-	140	67	47.9	28	20.0	8	5.7
51-	67	29	43.3	10	14.9	4	6.0
61-	27	9	33.3	6	22.2	1	3.7

also used. Recently some of the young adult residents have left their farms to start working either in commercial enterprises or to enter factories.

Prevalence of hookworm and other intestinal nematode infections

The prevalence of ascariasis, trichuriasis and hookworm infection is high in this Anhui village. As shown in Table 1, the overall prevalence of intestinal nematodiases was 54.1% in Zhongzhou village (Nanlin County), corresponding to prevalences of 33.2%, 25.0% and 9.0%, for hookworm, ascariasis and trichuriasis, respectively. Both males and females had a similar prevalence for *Ascaris* and *Trichuris*, although males had a significantly higher rate of hookworm infection (37.9%) compared to females (26.7%) ($p < 0.05$).

The relationship between age and prevalence of the three intestinal nematode infections is presented in Table 2 and Fig 2, and showed distinct patterns for ascariasis and trichuriasis compared to hookworm infection. For ascariasis the prevalence was greatest in children between 2-10 years of age, while adolescents between the ages of 11-20 had the highest prevalence of trichuriasis. The prevalence of these two nematodiases declined significantly in adulthood. In contrast, the prevalence of hookworm showed a distinctly different pattern, with the highest rate of infections among middle aged adults between the ages of 31-50. There was a modest decline in the hookworm prevalence among the elderly over the age of 61 years.

Hookworm intensity and species

Infection intensity was determined only for hookworm and was estimated on the basis of quan-

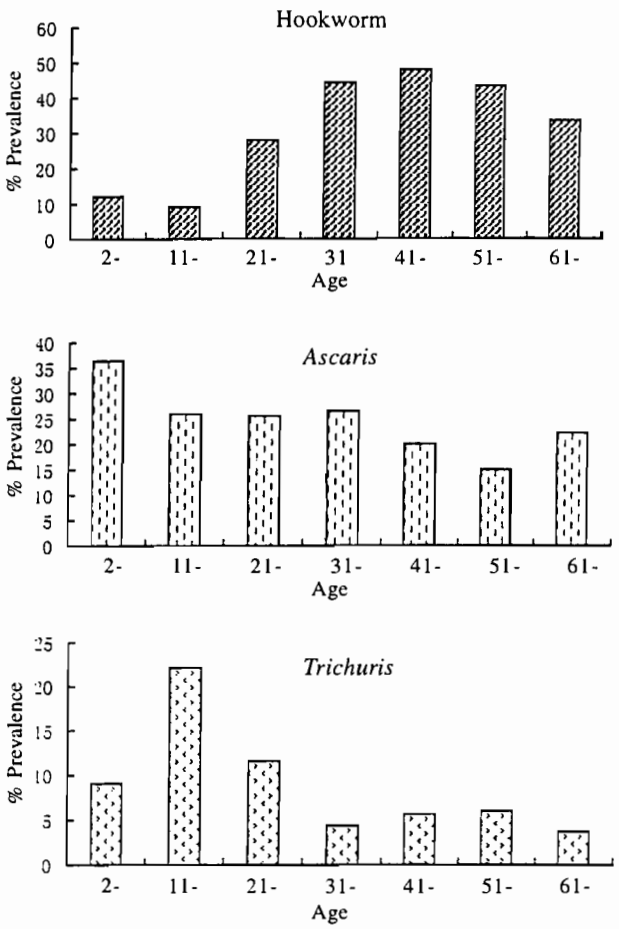

Fig 2–The relationship between the prevalence of three helminths and ages in pilot site of Zhongchou village.

Table 3
Collection of hookworm, *Ascaris* and *Trichuris* from feces within 48 hours after treatment of the subjects with quantrel.

Species of parasite	No. of examined	No. of positive	Vermifuge rate(%)	No. of worm	Mean worm per subject
Hookworm	99 (L)	48	49.5	271 (Ad 255 +Na 16)	5.6
	23 (M)	16	69.6	111 (Ad 109 +Na 2)	6.9
	1 (H)	1	-	18 (Ad)	
Ascaris	23	19	82.6	90	4.7
Trichuris	40	3	7.5	4	1.3

L: light; M: mederate; H: heavy. Ad: *Ancylostoma duodenale*; Na: *Necator americanus*.

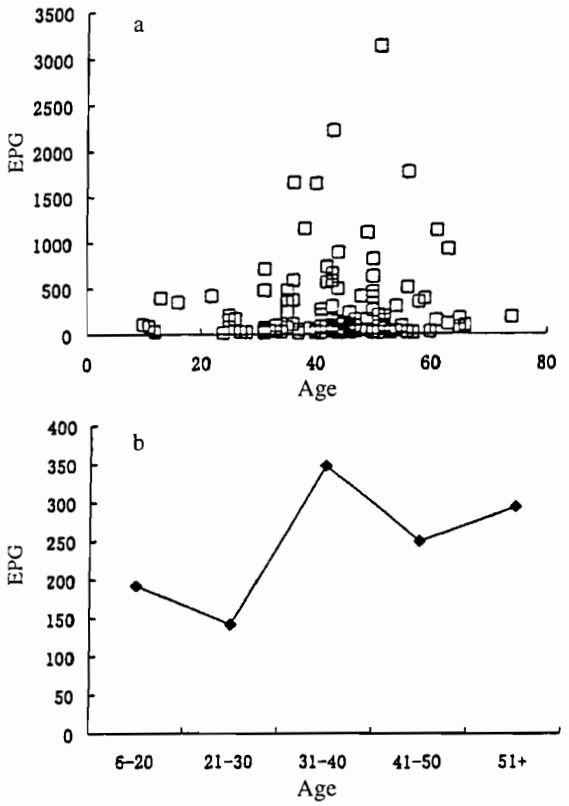

Fig 3–The intensity of hookworm infection in the residents expressed by the scatter EPG in each age group (a), and the relationship between mean EPG and age (b).

titative egg counts. Light (80.5%; 99/123) and moderate (18.7%; 23/123) hookworm infections predominated in this region. Only one (0.8%; 1/123) of the residents harbored a heavy hookworm infection with 3,132 EPG. The overall mean EPG was 277. Fig 3 shows the intensity of hookworm infection as a function of age and is represented both by individual EPG in a scatter plot (Fig 3a) as well as by age categories (Fig 3b). Hookworm intensity peaks in the residents aged 31-40 with a gradual decline subsequently.

Ancylostoma duodenale was the predominant hookworm found in Zhongzhou, as determined by morphology of L_3 recovered from feces by coproculture. 84 out of 123 residents who showed larva positive, 71 were noted to harbor pure *A. duodenale* infection, while 12 harbored mixed infections with *A. duodenale* and *N. americanus*. Only one resident had a pure *N. americanus* infection. The ratio of *A. duodenale* L_3 to *N. americanus* L_3 was determined to be 35:1.

To confirm the species of hookworm recovered from subjects living in the village, a total of 123 subjects were treated orally with quantrel and the adult worms were recovered after 48 hours of fecal collection. As shown in Table 3, 271 adult hookworms were recovered from 48 (49.5%) of the 99 residents with light hookworm infection (mean of 5.6 adult hookworms per subject), of which 94% were *A. duodenale*. It was determined that 98% of the 111 adult hookworms recovered from moderately infected residents (mean of 6.9 adult hookworms per subject) were *A. duodenale*, while 18 adult *A. duodenale* hookworms were recovered from the one heavily infected subject (Table 3). Overall, 400 adult hookworms were recovered with an *A. duodenale* to *N. americanus* ratio of 21:1.

DISCUSSION

These studies indicate that hookworm and other intestinal nematode infections have remained highly prevalent in this rural village in Anhui Province, since they were studied as part of the nationwide

intestinal parasite survey. The prevalence of hookworm in Zhongzhou Village is similar to that reported overall for Anhui Province approximately a decade ago, although the prevalence, of ascariasis and trichuriasis has diminished although village specific data are not available for Zhongzhou. Hookworm infection in this region is still associated with the reliance of human feces as fertilizer for the major cash crops in this area, particularly cotton and tobacco. The higher prevalence among males is reflective of hookworm infection as an occupational disease among these workers.

The hookworm infections were predominantly light as determined by quantitative hookworm egg counts and recovery of adult hookworms after anthelmintic chemotherapy, and were comparable to the light intensity which we observed recently in Jiangsu Province (Sun *et al*, 1999). Jiangsu Province borders Anhui Province to the east. *Ancylostoma duodenale* was noted to be the predominant hookworm in Anhui with ratios of *A. duodenale* to *Necator americanus* of 35:1 and 21:1 when determined by L_3 or adult hookworm recovery and morphology, respectively. High ratios of *A. duodenale* to *N. americanus* were also noted to occur in the northern part of neighboring Jiangsu Province (Sun *et al*, 1999). This species ratio is reversed in the southern Chinese province of Hainan Island in the South China Sea where *N. americanus* is the predominant, or in some cases the exclusive hookworm (Chen *et al*, 1999). It has been suggested that *A. duodenale* may survive in the colder temperatures that occur in more northerly latitudes of Anhui and Jiangsu during the Winter months, whereas *N. americanus* cannot, possibly because of the ability of the former to undergo arrested development in human tissues during times of adverse seasonal conditions (Schad, 1990). We did not attempt to look at human hookworm egg excretion during different times of the year in this region.

We noted previously that the prevalence of *N. americanus* both in Hainan and Sichuan Provinces increased steadily with age, with r coefficients of 0.96-0.97 (Chen *et al*, 1999; Liu *et al*, 1999). This trend for *Necator* infections has been noted by other investigators (Bundy, 1990; Humphries *et al*, 1997; Crompton, 1998). In Anhui, both the prevalence and intensity of *A. duodenale* exhibited a peak in middle aged residents between the ages of 31-50 with a subsequent decline among the elderly. However throughout this village the majority of *A. duodenale* infections were light or moderate, so that the variation between residents was not high. This is in contrast to Hainan where heavy infections predominated (Chen *et al*, 1999). We are determining whether the decline in hookworm intensity after the age of 40 years correlates with the appearance of antibodies directed against *Ancylostoma* L_3 antigens. This could determine whether it might be possible to administer chemically defined *Ancylostoma* antigens as a vaccine in order to reduce host worm burdens among exposed populations (Hotez *et al*, 1996; Xiao *et al*, 1998a, 1998b, 1999; Yang *et al*, 1998; Zhan *et al*, 1999).

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