# EPIDEMIOLOGY OF HUMAN GEOHELMINTH INFECTIONS (ASCARIASIS, TRICHURIASIS AND NECATORIASIS) IN LUSHUI AND PUER COUNTIES, YUNNAN PROVINCE, CHINA

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Abstract. Between April and June of 1998, the prevalence and intensity of geohelminth infections caused by hookworm, Ascaris and Trichuris were investigated in two rural Yunnan villages. In Liuku, a village of Lisu indigenous people in Lushui County, there was an overall geohelminth prevalence of 72% (48%, 43% and 16% for hookworm infection, ascariasis, and trichuriasis, respectively). The prevalence of ascariasis was greatest among preschool and school aged children, whereas the prevalence of trichuriasis was greatest among teenagers and the prevalence of hookworm increased until the age of 10-15 and then remained high throughout adulthood. In Linger, a village of Han Chinese, located in Puer County, there was an overall geohelminth prevalence of 77% (30%, 60% and 36% for hookworm infection, ascariasis, and trichuriasis, respectively). The differences in prevalence for hookworm and ascariasis were statistically significant. The prevalence of hookworm in Linger increased steadily with age and did not plateau, but there were no discernible patterns of prevalence versus age for either ascariasis or trichuriasis. Heavy trichuriasis infections were noted to occur in Linger. In both villages, more than 98% of the hookworm infections were of light and moderate intensity. Both by morphologic identification of third-stage infective larvae (L3) from eggs as well as identification of adult hookworms recovered from adult residents after treatment with quantrel, Necator americanus was identified as the exclusive hookworm in each village. Geohelminth infections caused by Ascaris, Trichuris and hookworm remain highly endemic to the rural areas of Yunnan Province in southwestern China.

## INTRODUCTION

Geohelminth infections caused by Ascaris, Trichuris and hookworm are highly endemic to south China. Based on a nationwide survey of intestinal parasites completed in 1992 it was determined that 531 million, 212 million and 194 million individuals harbor these three geohelminths, respectively (Yu et al, 1994; Hotez et al, 1997). Since 1997 we have been reinvestigating the epidemiology of geohelminth infections in the major endemic regions of China including Anhui, Hainan, Jiangsu, and Sichuan Provinces (Sun et al, 1998; Liu et al, 1999; Wang et al, 1999). Our studies show that in regions where economic progress has been rapid, such as in Jiangsu Province, there has been a precipitous decline in the prevalence and intensity of geohelminth infections, whereas in

other regions the prevalence and intensity has remained the same or has even increased.

Yunnan Province in southwestern China shares borders with Myanmar, Lao PDR and Vietnam, and is comprised of both mountainous and low-lying subtropical regions. As part of the nationwide survey Yunnan was found to be highly endemic for geohelminth infections, with prevalence rates of Ascaris, Trichuris, and hookworm infections of 59.6%, 27.3% and 19.3%, respectively. This previous study was based on 103 pilot sites comprising 53,061 individuals between 1987 and 1991. Among the environmental risk factors reported for geohelminth transmission in Yunnan Province were the use of human feces as fertilizer as well as rainfall and temperature. In addition, high rates of infection inversely correlated with elevation. Here we report the epidemiology of geohelminth infections in two representative villages in Yunnan, one in a low-lying subtropical region and the other in a mountainous area, when they were re-investigated in April and June of 1998 - 7 years after completion of the nationwide survey.

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## MATERIALS AND METHODS

## Sample selection and fecal examination

Fecal examinations were performed on 304 local residents of Liuku village (Lushui County) and 462 local residents of Linger village (Puer County) between April and June of 1998. Ethical clearance for these studies were obtained both from the Office of Research Protection of the National Institutes of Health and by Protocol 10453 of the Yale University School of Medicine IRB. Identification of geohelminth eggs were determined by a thick smear using the Kato-Katz method (Katz et al, 1972). Quantitative hookworm egg counts were also determined 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 (L3) which were reared from eggs (Wu and Peng, 1985; Ren et al, 1994). From each patient, 200 L3 were examined.

## Antihelmintic treatment and adult worm recovery

In the two sites, 25 and 28 hookworm-infected adult residents of Liuku and Linger, respectively, were treated orally with quantrel (each tablet containing 100 mg pyrantel base and 100 mg oxantel base) at a total dose of 1,000 mg of each drug (10 tablets). The first five tablets were administered in the morning and the remaining 5 tablets were given the following day. Following anthelmintic treatment, all feces from each patient were collected for 48 hours, and washed through sieves with water. The adult hookworms were individually picked by careful examination of the sediment, and worm number was recorded.

## RESULTS

#### General characteristics of the study sites (Fig 1)

Lushui County is affiliated to Nujiang Prefecture is located in the mountainous region of southwestern Yunnan Province. Liuku villiage is in southern Nujiang, situated in a valley east of Gaoligong Mountain near the Nujiang River (Lat 25°51; Long 98°51; Elevation 860 m). The average annual temperature and rainfall are 20°C and 11 cm, respectively. The residents of Liuku village

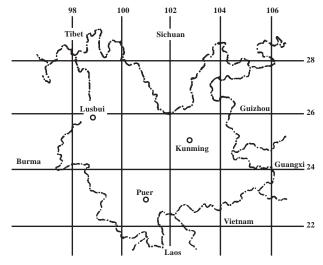


Fig 1–Map of geographical distribution of hookworm infection in Lushui and Puer County, Yunnan Province.

belong to the indigenous Lisu Nationality. Puer County is in the Simao Prefecture and located in forested hills in southern Yunnan Province. Linger village is in the center of Simao (lat 23°04; long 101°02, elevation 1,320 m). The average annual temperature and rainfall are 18°C and 13.98 cm, respectively. The residents of Linger village are Han Chinese. Both sites are subtropical and intimately associated with agricultural pursuits, especially for the cultivation of vegetables including corn, potatoes and sweet potatoes.

#### Prevalence of geohelminth infections

As shown in Table 1, the overall prevalence of geohelminth infections was 72% in Liuku village (Lushui County), corresponding to prevalences of 48%, 43%, and 16% for hookworm infection, ascariasis, and trichuriasis, respectively. The overall prevalence of geohelminth infections in Linger village (Puer County) was 77%, corresponding to 30%, 60% and 36% for hookworm infection, ascariasis, and trichuriasis, respectively. Compared to Linger, Liuku had a higher prevalence of hookworm ( $\chi^2 = 25.91$ ), but a lower prevalence of ascariasis ( $\chi^2 = 20.68$ ) and trichuriasis ( $\chi^2 = 34.23$ ). For both villages combined, females had a higher prevalence of ascariasis and trichuriasis than males, but the prevalence of hookworm was similar for either sex (Table 2). The relationship between age and prevalence of the three geohelminth infections

| Pilot sites | No.<br>examined |     | ve with<br>ninths | Hool | worm | As  | caris | Trici | huris |
|-------------|-----------------|-----|-------------------|------|------|-----|-------|-------|-------|
|             |                 | No. | %                 | No.  | %    | No. | %     | No.   | %     |
| Liuku       | 304             | 219 | 72.0              | 146  | 48.0 | 132 | 43.4  | 48    | 15.8  |
| Linger      | 462             | 357 | 77.3              | 138  | 29.9 | 278 | 60.2  | 165   | 35.7  |
| Total       | 766             | 576 | 75.2              | 284  | 37.1 | 410 | 53.5  | 213   | 27.8  |

Table 1 The prevalence rate of helminths in two villages.

|     |             |      |    | Table     | 2  |      |     |        |            |
|-----|-------------|------|----|-----------|----|------|-----|--------|------------|
| The | prelvalence | rate | of | helminths | in | male | and | female | residents. |

| Sex    | No.<br>examined |     | ve with<br>ninths | Hool | worm | As  | caris | Tric | huris |
|--------|-----------------|-----|-------------------|------|------|-----|-------|------|-------|
|        |                 | No. | %                 | No.  | %    | No. | %     | No.  | %     |
| Male   | 299             | 198 | 66.2              | 111  | 37.1 | 139 | 46.5  | 67   | 22.4  |
| Female | 467             | 378 | 80.9              | 173  | 37.0 | 271 | 58.0  | 146  | 31.3  |
| Total  | 766             | 576 | 75.2              | 284  | 37.1 | 410 | 53.5  | 213  | 27.8  |

Table 3

The relationship between the prevalence rate of three helminths and the ages of residents in pilot site of Liuku village and Linger village.

| Age (years) | No.<br>examined | Hool | kworm | Ase | caris | Trick | huris |
|-------------|-----------------|------|-------|-----|-------|-------|-------|
|             |                 | No.  | %     | No. | %     | No.   | %     |
| 0-          | 24              | 2    | 8.3   | 16  | 75.0  | 3     | 12.5  |
| 5-          | 54              | 11   | 20.4  | 26  | 48.2  | 16    | 29.6  |
| 10-         | 52              | 14   | 26.9  | 30  | 57.7  | 18    | 34.6  |
| 15-         | 31              | 10   | 32.3  | 19  | 61.3  | 8     | 25.8  |
| 20-         | 143             | 56   | 39.2  | 77  | 53.9  | 30    | 21.0  |
| 30-         | 137             | 51   | 37.2  | 72  | 52.6  | 39    | 28.5  |
| 40-         | 190             | 75   | 39.5  | 113 | 59.5  | 62    | 32.6  |
| 50-         | 83              | 42   | 50.6  | 37  | 44.6  | 20    | 24.1  |
| 60≥         | 52              | 23   | 44.2  | 18  | 34.6  | 17    | 32.7  |
| Total       | 766             | 264  | 37.1  | 410 | 53.5  | 213   | 27.8  |

in Liuku village is presented in Fig 2. The prevalence of hookworm infection increased with age until the age of 10-15 when it subsequently reached a plateau. In contrast, the prevalence of ascariasis was at its maximum in preschool and school-aged children. The prevalence of trichuriasis was greatest for teenagers. The relationship between age and prevalence of the three geohelminth infections was different in Linger (Fig 3), whereas the prevalence of hookworm infection increased with age and did not plateau. In addition there were no discernible patterns of age versus prevalence for either ascariasis or trichuriasis. When data from the two villages were combined (Table 3), significant trends

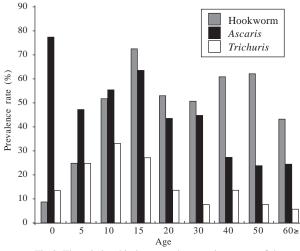


Fig 2–The relationship between the prevelence rate of three helminths and the ages of residents in pilot site of Liuku (Lushui County).

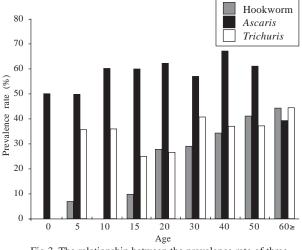


Fig 3–The relationship between the prevelence rate of three helminths and the ages of residents in pilot site of Linger (Puer County).

were noted for hookworm infection, which appeared to rise linearly with age (r = 0.875), whereas the prevalence of ascariasis decreased with age (r = -0716). The highest prevalence of hookworm was noted in adults over the age of 50, while the lowest prevalence of ascariasis occurred in this age group. As shown in Table 4, polyparasitism with more than a single geohelminth was common. In Liuku village 42.5% of the residents examined harbored more than one geohelminth, while in Linger village 48.7% of the residents had multiple parasites.

#### Hookworm intensity and hookworm species

Geohelminth infection intensity based on EPGs was determined only for hookworm and Trichuris infections. As shown in Table 5, the two villages showed differences in the intensity of their endemic hookworm. In Liuku, 56% and 42.5% of the infected residents harbored light and moderate infections, respectively. In Linger, 71% and 27.5% of the infected residents harbored light and moderate infections, respectively. At each site only 2 residents harbored heavy infections. All of the hookworm infected residents harbored pure infections with Necator americanus as determined by recovery and identification of L3 reared from eggs. To confirm this observation, the adult hookworms were recovered from a total of 53 residents each of whom were treated with quantrel. The total number of adult hookworms recovered from 25 Liuku residents was 562, while 216 adult hookworms were recovered from 28 Linger residents. All of these adult hookworms were identified by morphological criteria as N. americanus. As shown in Table 6. Light trichuriasis infections predominated in Liuku whereas moderate and heavy infections predominated in Linger.

| Pilot<br>site | No<br>examined |         | Residents infected with 1, 2 or 3 species of helminths |       |      |       |      |       |  |
|---------------|----------------|---------|--|-------|------|-------|------|-------|--|
| Site          | exumited       | miceteu | 1 sp   | ecies | 2 sp | ecies | 3 sp | ecies |  |
|               |                |         | No.  | %     | No.  | %     | No.  | %     |  |
| Liuku         | 304            | 219     | 126  | 57.5  | 81   | 37.0  | 12   | 5.5   |  |
| Linger        | 462            | 357     | 176  | 49.3  | 116  | 32.5  | 65   | 18.2  |  |
| Total         | 766            | 576     | 302  | 52.4  | 197  | 34.2  | 77   | 13.4  |  |

 Table 4

 Residents infected with 1, 2 or 3 species of intestinal helminths in two villages.

Table 5 Determination of egg number in each gram of feces (EPG) in 284 residents with hookworm infection.

| Pilot site | No.<br>examined — |            | EPG           |            |
|------------|-------------------|------------|---------------|------------|
|            | examined          | <400 (%)   | 400-3,000 (%) | >3,000 (%) |
| Liuku      | 146               | 82 (56.2)  | 56(38.4)      | 8 (5.5)    |
| Linger     | 138               | 98 (71.0)  | 30(21.7)      | 10(7.3)    |
| Total      | 284               | 180 (63.8) | 86(30.3)      | 18 (6.3)   |

Table 6 Determionation of egg number in each gram of feces (EPG) in 213 residents with trichuriasis.

| Pilot site | No.<br>examined — | EPG       |                 |             |  |  |  |
|------------|-------------------|-----------|-----------------|-------------|--|--|--|
|            |                   | 1-999 (%) | 1,000-9,999 (%) | >10,000 (%) |  |  |  |
| Liuku      | 48                | 47(97.8)  | 1 (2.1)         | 0 (-)       |  |  |  |
| Linger     | 165               | 40(24.2)  | 115 (69.7)      | 10(6.1)     |  |  |  |
| Total      | 213               | 87(40.8)  | 116 (54.5)      | 10 (4.7)    |  |  |  |

## DISCUSSION

These studies indicate that geohelminth infections caused by Ascaris, Trichuris and hookworm remain highly prevalent in Yunnan since they were last investigated between 1987 and 1991 as part of the nationwide survey on intestinal parasites. The overall prevalence of geohelminth infections exceeds 70% and are among the highest in China. The geohelminth prevalences in Linger were found to be approximately the same as they were in 1977 (62% ascariasis, 31% trichuriasis, and 66% hookworm). Similar data are not available for Liuku. In Liuku, hookworm and Ascaris were the predominant geohelminths with a low prevalence of Trichuris. In contrast, the residents of Linger had a prevalence of 30% or more of all three geohelminths, with Ascaris being predominant. The lower prevalence of hookworm in Linger may reflect its higher elevation and relatively dry conditions, which are unfavorable for hookworm larval transmission. However, we cannot rule out differences in behavior some of which may arise because of differences in ethnic background.

In Liuku, *Ascaris* and *Trichuris* exhibited the highest prevalence in children with a significant decrease associated with entrance into adulthood. This pattern has been seen previously for intensity

patterns of *Ascaris* and *Trichuris* infections in other parts of the world (Crompton, 1998; Bundy 1990). We have also observed this pattern in Hainan Province (unpublished observations) in the South China Sea. In contrast, the residents of Linger exhibited no identifiable pattern of age-associated prevalence for either ascariasis or trichuriasis.

Among the hookworms, Necator was observed to be the exclusive hookworm in both Yunnan villages. This organism has also been described as the predominant hookworm in other parts of Southeast Asia including Vietnam (Humphries et al, 1997) and Hainan (unpublished observations), whereas A. duodenale becomes more frequent at more northerly Asian latitudes (Hotez et al, 1997). In contrast to the age-associated prevalence patterns observed for Ascaris and Trichuris, the prevalence of hookworm increased with age peaked among the elderly. Moderate infections predominated among the elderly residents. We are looking into geohelminth transmission patterns to understand why the elderly are so susceptible to Necator infections. The age-associated rise in Necator infections may have a biological or immunological basis, but we cannot rule out the possibility that the social structure of this developing economy strongly influences the prevalence, intensity and species-predominance. For instance, it was shown

previously that the use of human feces for fertilizer is associated with increased intensity of *Necator* infections among Vietnamese women (Humphries *et al*, 1997). We are investigating this link in Yunnan as well. We have observed that while most of the female residents are engaged in agricultural pursuits (cultivation of rice, peanuts, sweet potatoes and other vegetables), some of the young male adult residents have recently started to leave agricultural pursuits in favor of commercial enterprises or to enter factories, leaving elderly residents to work in the fields. This observation may explain some of the sex and age differences observed in our study.

## ACKNOWLEDGEMENTS

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