Comparison of the structures of natural and re-established populations of *Ascaris* in humans in a rural community of Jiangxi, China

W. PENG[1]*, X. ZHOU[2] *and* X. CUI[2]

[1] *Jiangxi Medical Science Research Institute, Nanchang 330006, P. R. China*
[2] *Department of Parasitology, Jiangxi Medical College, Nanchang 330006, P. R. China*

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SUMMARY

To compare the structures of natural and re-established populations of *Ascaris* in humans, universal (mass) chemotherapy was carried out at the beginning and the end of the study year using pyrantel pamoate. Worms expelled within 48 h of treatment were collected, their sex determined, and measurements made of length, width and weight. Length was used as the criterion for estimating the developmental stage of the worms. In comparison with the natural population, the re-established population displayed similar sex ratio as well as distribution patterns among individuals and age groups of the host. However, the mean worm burden of the re-established population was significantly decreased, with a reduction of burdens in children aged 5–9 years. Also, the re-established population showed significant changes in population structure and worm measurements in that it comprised more immature and less fertile males, less fertile and more senile females, smaller and lighter males, larger (but not heavier) females than the natural population. The results suggested that the re-established *Ascaris* population did not restore to its original status in relation to mean density, composition and fecundity. Therefore, universal treatment once a year should decrease the transmission of *Ascaris* in humans. Combined with previous results for the same study sites, the present findings also indicated that caution is warranted to avoid misleading conclusions when using prevalence and faecal egg counts as parameters for evaluating the success of control programmes.

Key words: *Ascaris lumbricoides*, prevalence, intensity, chemotherapy, China.

INTRODUCTION

Ascaris lumbricoides is widely distributed in over 150 territories of the world (Crompton, 2001). The number of people infected is estimated at 1·4–1·5 billion (Chan *et al*. 1994; O'Lorcain & Holland, 2000), but the data may be inadequate for some developing countries due to deficient sampling and diagnostic procedures (Crompton, 2001). Also, the techniques of faecal examination may underestimate the actual prevalence (Guyatt & Bundy, 1993). *Ascaris lumbricoides* is found in all provinces of China, and the total number of infected people has been estimated at > 530 million, which represents $\sim 47\%$ of the overall prevalence according to a nation-wide survey of > 1 million people from approximately 2850 locations in this country (Yu, 1992; Peng, Zhou & Crompton, 1998).

A number of studies have compared the prevalence and intensity of *Ascaris* infection in humans before and after anthelminthic treatment. Most of them were aimed at evaluating anthelminthic efficacy or different treatment strategies (e.g. Thein Hlaing, Than Saw & Myint Lwin, 1987; Sinniah, Chew & Subramaniam, 1990; Albonico *et al*. 1994; Holland *et al*. 1996; Rahman, 1996). However, only a few studies have attempted to investigate the impact of treatment on the structures of *Ascaris* populations (e.g. Seo, Cho & Chai, 1980; Elkins & Haswell-Elkins, 1989), which is central to the development and monitoring of effective control programmes. In spite of the accepted public health significance of *A. lumbricoides* (O'Lorcain & Holland, 2000), there remains a paucity of information on these aspects. The aim of this study was to compare the population structure of *Ascaris* in humans before and after treatment (using a 1 year interval) in 2 adjacent villages in South-east China, in order to gain a better understanding of the population biology of *A. lumbricoides*, as a foundation for chemotherapeutic control of human ascariasis.

* Corresponding author (present address): Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia. Tel: +61 3 9731 2283. Fax: +61 3 9731 2366. E-mail: weidongp@unimelb.edu.au

MATERIALS AND METHODS

Relevant background information on the study

This study is an extension to a previous research project, where details relating to the study area were reported (Peng *et al*. 1996, 1998). In brief, 2 adjacent villages, Laochi and Panchi, approximately 40 km from Nanchang, the capital city of Jiangxi Province,

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China, with similar human population structure, socio-economic status and cultural habits, were selected as the study sites. Before the first universal treatment, a 1-year longitudinal investigation (consisting of 6 cross-sectional surveys, conducted at 2-monthly intervals) on the transmission of *A. lumbricoides* had been carried out using the Kato-Katz technique (WHO, 1991). The first universal treatment was given immediately after the sixth cross-sectional survey. Another 5 cross-sectional surveys followed in the second year. The second universal treatment was given immediately after the final survey. For each survey, no statistical difference was found in the prevalence or the mean faecal egg counts (in eggs per gram, EPG) between the 2 villages (Peng *et al*. 1996; Peng & Zhou, 2001). For simplicity, we designate the *Ascaris* population expelled by the first treatment as the 'natural population', because no prior chemotherapeutic intervention had been used for ascariasis control in the study areas (Peng *et al*. 1996), and the worm population expelled by the second treatment as the 're-established population'.

Chemotherapy and assessment of drug efficacy

Using pyrantel pamoate (Jiangxi Pharmaceuticals, 10 mg/kg, 1 dosage), 2 universal treatments were given in July 1994 and July 1995 respectively. On the day of treatment, each household was visited by members of the research team and local doctors. Totally 466 and 398 people were treated respectively with a correct dosage taken within 1–3 h before sleep. After medication, 2 or 3 plastic bags marked with the identification number and name were given to the treated person for faecal collection during the next 2 days. People who were having other medicines and the pregnant women as well as young children (below 6 months) did not accept the treatment. For this anthelminthic drug, a cure rate of $> 90\%$ has been reported (see Crompton, 2001). Faecal samples were collected for 48 h after treatment to obtain *Ascaris* worms. To examine the drug efficacy, faecal egg counts were conducted on the tenth day with the Kato-Katz technique after each treatment. Two slides for each faecal sample were prepared and observed, and the mean egg counts for each faecal sample were obtained (see Peng *et al*. 1996). The prevalence and EPG post-treatment were determined in order to compare with pre-treatment values.

Collection of raw data

Ascaris worms from individual persons were washed and fixed in 5% formalin. Worms were counted and their sex determined. Measurements of length, width and weight were made for each worm. Worm length was used as the criterion for estimating the developmental stage of a worm, essentially according to Seo (1990). In brief, females with a length of < 125 mm and males of < 105 mm were recorded as immature worms; otherwise, they were recorded as adults. Females with a length of 125–135 mm and males of 105–115 mm were both recorded as fertile (within the pre-patent period of infection). Females with a length of 135–250 mm and males > 115 mm were recorded as fertile and fecund. Females with lengths of > 250 mm were recorded as those whose egg-laying capacity was decreasing, or senile worms.

Statistical methods

The Chi-square test was used to compare frequency, and one-way analysis of variance (ANOVA) to compare the means. Raw data were transferred logarithmically before using ANOVA. Spearman's (non-parametric) rank correlation test was used as required. The SPSS statistical software was used to analyse the data.

RESULTS

Ascaris worms were obtained from 222 and 198 infected persons from the first and second universal treatments, respectively. The mean worm burden was calculated as the number of worms from the infected persons rather than from all treated people. Since the 2 villages showed similar baseline data for the transmission of ascariasis (Peng *et al*. 1996, 1998; Peng & Zhou, 2001), and there was no significantly statistical difference in worm burdens (means ± S.D.) between the 2 villages for each treatment (first treatment: $6{\cdot}95 \pm 7{\cdot}46$ *vs* $9{\cdot}93 \pm 13{\cdot}70$, $F = 1{\cdot}39$, D.F. $= 1$, $P = 0{\cdot}240$; second treatment: $5{\cdot}44 \pm 5{\cdot}84$ *vs* $7{\cdot}09 \pm 11{\cdot}10$, $F = 0{\cdot}06$, D.F. $= 1$, $P = 0{\cdot}801$), the data representing both villages were pooled for further analysis.

Assessment of drug efficacy

Of 466 treated people, 123 were examined for drug efficacy after the first treatment. The average prevalence decreased from 72·1% (pre-treatment) to 4·1% (post-treatment), which represented a cure rate (CR) of 94·3%. The mean EPG decreased from 15961 to 3303, representing an egg reduction rate (ERR) of 79·3%. After the second treatment, of 398 treated people, a total of 187 were examined. The CR and ERR were determined to be 95·1% and 98·2%, respectively.

Distribution of Ascaris *among individual infected humans*

The ratios of variance to mean of the worm burden for both natural and re-established populations were >1 (1·33 *vs* 1·45). When using the value of mean worm burden as a threshold, it was found, at the first treatment, that 70·6% of infected individuals

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Table 1. Worm burdens (means ± S.D.) of the natural and re-established populations of *Ascaris lumbricoides* according to host age

(Numbers of people of each age group given in parentheses after the mean burden.)

Age groups	Natural	Re-established	*P* (*F*) value
0–4	8·61 ± 6·19 (38)	9·21 ± 10·57 (34)	0·533 (0·39)
5–9	15·08 ± 13·24 (39)	7·85 ± 9·16 (39)	0·011 (6·80)*
10–14	11·41 ± 15·99 (29)	5·91 ± 5·78 (23)	0·281 (1·19)
15–24	6·46 ± 8·57 (26)	1·92 ± 1·51 (12)	0·017 (6·32)*
25–34	3·48 ± 2·53 (29)	4·33 ± 3·76 (21)	0·50 (0·46)
35–44	4·17 ± 4·41 (12)	2·56 ± 2·94 (16)	0·277 (1·23)
45–54	8·24 ± 16·31 (25)	5·36 ± 8·78 (14)	0·377 (0·80)
⩾ 55	4·29 ± 7·63 (14)	9·50 ± 21·37 (10)	0·538 (0·39)
Overall	8·64 ± 11·49 (212)	6·39 ± 9·19 (169)	0·0085 (6·98)

* Statistical significance ($P < 0·05$).

Table 2. Structure of natural and re-established populations of *Ascaris lumbricoides*

	Worm numbers		
	Natural	Re-established	*P* (χ^2) value
Male/female	811/1426 (0·569)	469/756 (0·620)	0·236 (1·40)
Immature worms/all worms	129/2237	76/1225	0·602 (0·27)
Immature males/all males	16/811	28/469	0·000 (14·30)*
Prepatent males/all males	28/811	21/469	0·357 (0·85)
Fertile males/all males	767/811	420/469	0·001 (11·13)*
Immature females/all females	113/1426	48/756	0·181 (1·79)
Prepatent females/all females	44/1426	24/756	0·909 (0·01)
Fertile females/all females	1058/1426	516/756	0·003 (8·67)*
Senile females/all females	211/1426	168/756	0·000 (18·98)*

* Statistical significance ($P < 0·05$).

harboured less worms than the mean burden, accounting for < 26·2 % of the total worm population. Hence, 73·8 % of the natural worm population was distributed among approximately 30 % of the people. The re-established *Ascaris* population retained a similar distribution pattern in that around 70 % of the worm population was distributed in 25·8 % of the people whose worm burden was greater than the mean burden, and 74·2 % of the people with a worm burden of less than the mean worm burden harboured only about 30 % of the re-established *Ascaris* population. Moreover, the female and male worms were also found to have similar distribution patterns when the mean burden representing each sex was used as the threshold.

Distribution of Ascaris *among different gender and age groups of humans*

No significant difference in worm burdens between male and female host individuals was detected ($F =$ 0·05–1·43, D.F. = 1, $P =$ 0·234–0·829) for both treatments. However, among different host age-groups, significant variation was detected for the natural population ($F = 5·77$, D.F. = 7, $P = 0·000$) and the re-established population ($F = 3·60$, D.F. = 7, $P =$ 0·0013). The re-established population showed a significant decrease in mean worm burden, and the human age-group profile showed that this decrease coincided with a significant reduction in worm burdens for humans aged 5–9 and 15–24 years (Table 1). Analysis of the worm profile according to sex showed that the decrease was mainly the result of a reduction in the number of females (5·20 ± 7·58 *vs* 3·60 ± 4·97, $F = 5·91$, D.F. = 1, $P = 0·015$) rather than a reduction of males (2·81 ± 4·03 *vs* 2·22 ± 3·80, $F = 3·79$, D.F. = 1, $P = 0·052$).

Structure of the natural and re-established populations of Ascaris

While the natural and re-established populations were found to possess similar sex ratios (0·569 *vs* 0·620) and percentages of immature or adult worms, some changes were determined in population structure in terms of the percentages of immature and fertile males, fertile and senile females (Table 2).

Ascaris *measurements in relation to worm sex*

Compared with the natural population, the mean length and weight of male *Ascaris* in the re-

Table 3. Measurements (means ± S.D.) of *Ascaris lumbricoides* from the natural and re-established populations in relation to worm sex

Worms (*n*)	Length (mm)	Width (mm)	Weight (g)
Males			
Natural (811)	156·4 ± 2·7	3·5 ± 0·8	1·52 ± 0·8
Re-established (469)	153·6 ± 2·9	3·4 ± 0·8	1·38 ± 0·6
P (*F*) value	0·032 (4·626)*	0·265 (1·246)	0·003 (9·005)*
Females			
Natural (1426)	200·8 ± 49·8	4·7 ± 1·5	3·48 ± 1·8
Re-established (756)	208·9 ± 49·2	5·0 ± 1·5	3·38 ± 1·7
P (*F*) value	0·001 (11·170)*	0·001 (10·683)*	0·775 (0·082)*

* Statistical significance ($P < 0{\cdot}05$).

Table 4. Measurements (means ± S.D.) of *Ascaris lumbricoides* from the natural and re-established populations in relation to worm burden

(Numbers of people/numbers of worms of each worm burden group given in parentheses after the mean weight.)

	Natural			Re-established		
Worm burden	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)	Weight (g)
1	184·7 ± 45·7	4·4 ± 1·5	2·80 ± 1·9 (45/45)	194·8 ± 58·8	4·3 ± 1·4	2·61 ± 1·7 (52/52)
2–5	161·0 ± 50·7	3·8 ± 1·4	2·26 ± 1·2 (83/258)	189·7 ± 37·2	4·5 ± 1·0	2·65 ± 1·2 (83/255)
6–10	181·7 ± 28·2	4·3 ± 0·8	2·84 ± 0·9 (34/242)	193·0 ± 22·0	4·4 ± 0·6	2·84 ± 0·7 (23/153)
11–14	168·5 ± 42·5	3·8 ± 1·0	2·45 ± 0·9 (19/233)	182·1 ± 24·5	4·2 ± 0·8	2·43 ± 0·7 (21/232)
⩾ 15	176·8 ± 27·8	4·1 ± 0·7	2·67 ± 0·8 (40/1092)	186·1 ± 29·5	4·4 ± 0·7	2·61 ± 0·8 (19/527)
P (*F*) value	0·016 (3·14)*	0·047 (2·46)*	< 0·0001 (166·9)*	0·933 (0·21)	0·678 (0·58)	< 0·0001 (148·2)*
Overall	172·5 ± 43·3	4·1 ± 1·2	2·6 ± 1·3 (221/1870)	190·2 ± 41·0	4·4 ± 1·1	2·63 ± 1·2 (198/1219)

* Statistical significance ($P < 0{\cdot}05$).

established population decreased significantly, but for females the significant increase in both mean length and mean width did not correlate with a significant increase in mean weight (Table 3). For both *Ascaris* populations, there was a significant divergence in measurements ($F = 343{\cdot}96–685{\cdot}94$, D.F. = 1, $P = 0{\cdot}000$) between male and female worms.

Ascaris *measurements in relation to worm burdens*

The relationship between worm burden and mean worm measurements (length, width and weight) was examined. No correlation was detected for both populations between each worm measurement and the worm burden by Spearman's correlation test ($r = -0{\cdot}009–0{\cdot}094$, $P = 0{\cdot}173–0{\cdot}904$), employing a standardization procedure to remove the age biases from individual records (see Haswell-Elkins, Elkins & Anderson, 1987). A comparison of measurements among 5 different worm burden groups was also carried out. Grouping was according to mean worm burdens for the 2 *Ascaris* populations and the previous classification of light and heavy infections based on worm counts (see Hall, Anwar & Tomkins, 1992). As shown in Table 4, larger and heavier worms were usually found in the groups with only 1 worm or with 6–10 worms (which represented approximately the mean worm burden from each treatment, see Table 1). Each measurement was found to be significantly different among the 5 different groups representing the natural population. However, for the re-established population, the length and width of worms reflected greater homogeneity, with no significant difference among groups being detected (Table 4).

Ascaris *measurements in relation to host sex and age*

There was no significant difference in worm measurements between male and female hosts for both treatments ($F = 0{\cdot}003–1{\cdot}171$, D.F. = 1, $P = 0{\cdot}955–0{\cdot}313$). However, all mean measurements were found to be significantly different among age groups of the host for both natural and re-established worm populations ($F = 2{\cdot}633–5{\cdot}083$, D.F. = 7, $P = 0{\cdot}013–0{\cdot}000$). Compared with the natural population, the worm length and weight of the re-established worms increased for most age groups, particularly for children, and significant differences were found in some age groups (see Table 5). A significant increase in mean length (Table 5) and mean width (4·1 mm *vs*

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Table 5. Length and weight (means ± S.D.) of *Ascaris lumbricoides* in relation to host age

(See Table 1 for the number of people in each age group.)

	Mean length (mm)			Mean weight (g)		
Age groups	Natural	Re-established	P (F) value	Natural	Re-established	P (F) value
0–4	145·7 ± 47·3	179·6 ± 37·3	0·002 (10·044)*	1·95 ± 1·0	2·28 ± 1·2	0·897 (0·017)
5–9	183·2 ± 30·8	203·2 ± 44·3	0·019 (5·721)*	2·71 ± 0·9	3·10 ± 1·0	0·042 (4·262)*
10–14	186·3 ± 32·4	202·5 ± 36·7	0·133 (2·332)	2·84 ± 1·3	2·88 ± 1·2	0·463 (0·547)
15–24	169·0 ± 46·5	154·8 ± 43·1	0·598 (0·283)	2·68 ± 1·4	1·49 ± 1·0	0·003 (10·176)*
25–34	176·2 ± 52·1	200·0 ± 39·2	0·092 (2·964)	2·65 ± 1·4	2·96 ± 1·3	0·308 (1·060)
35–44	174·1 ± 37·6	201·4 ± 24·8	0·034 (4·993)*	2·52 ± 1·4	2·59 ± 0·9	0·563 (0·344)
45–54	178·5 ± 33·1	170·7 ± 49·4	0·458 (0·562)	2·75 ± 1·2	2·30 ± 1·2	0·205 (1·661)
⩾ 55	163·3 ± 48·5	184·6 ± 35·3	0·241 (1·449)	2·11 ± 1·5	2·42 ± 1·2	0·416 (0·687)
Mean	172·5 ± 43·3	190·2 ± 41·0	< 0·001 (17·308)*	2·55 ± 1·3	2·63 ± 1·2	0·080 (3·087)

* Statistical significance ($P < 0·05$).

4·4 mm, see Table 4; $F = 10·135$, D.F. = 1, $P = 0·002$) was detected, but not in weight.

DISCUSSION

In this study, the efficacy of the 2 universal treatments was found to be similar, indicating that the comparison should be valid. Compared with the natural *Ascaris* population, the re-established population did not restore to its natural status after the first treatment, particularly in relation to mean worm burden, fecundity and population structure. This suggests that universal treatment once a year should be effective in reducing the transmission of ascariasis in humans.

Current strategies for chemotherapeutic control programmes are universal (mass), targeted and selected treatment regimens, with a limited numbers of anthelminthic drugs being recommended by the World Health Organization (see Crompton, 2001). However, for each regimen, a central issue is the treatment interval (O'Lorcain & Holland, 2000). It is clear that the more frequently the same drug is used, the more continual selection pressure is applied to the parasite population. It is proposed that 5–100 generations may be required before anthelminthic resistance would become evident in nematodes (Crompton, 2001). Therefore, in comparison with a 3-, 4- or 6- monthly treatment interval, the use of annual universal treatment may retard the development of drug resistance by reducing the selection pressure on the parasite.

In the present study, the re-established population had a significantly lower mean density, with a significant reduction in worm burdens in children aged 5–9 years. These results are distinct from a previous study (Thein Hlaing *et al*. 1987) which indicated that a 6-monthly universal treatment regimen reduced the infection intensity in both children and adults, but that 12-monthly treatments decreased the intensity only in adults. The sex ratio for the natural *Ascaris* population was similar to that of the re-established population (0·569 *vs* 0·620, $P > 0·05$), but distinctly different from a report from India (0·78 *vs* 0·91, $P < 0·05$, see Elkins & Haswell-Elkins (1989)). Also, the sex ratio values determined in the present study were different from those reported from Korea (0·74–0·82, see Seo (1990)) and the Philippines (0·83, see Monzon (1991) and 0·417, see Cabrera (1984)). Interestingly, there were significant differences within each sex in the percentages of immature, fertile and senile worms between the 2 populations. Irrespective of worm sex, the 'pooled' data showed that the re-established worms were significantly larger but not heavier. However, males and females displayed different patterns in that the males were shorter and lighter, and females larger but not heavier when compared with the natural population. That the increase in female size was not concomitant with an increase in weight may suggest a decrease in tissue density of the re-established females compared with those from the natural population, indirectly suggesting a lower fecundity in females. The findings of the present investigation contrast with a previous study (Elkins & Haswell-Elkins, 1989) which reported that male and female worms recovered after an 11-month period of re-infection were significantly longer and heavier than those expelled by initial treatment.

The homogeneity of worm length and width in the re-established population of *Ascaris* for different worm burden groups may relate to changes caused by universal chemotherapy, such as the release from the density-dependent limitation on resident worms (Anderson & May, 1985), and a weakening of previous concomitant immunity (Crompton, 1994). These changes may reduce the original heterogeneity among individual hosts, in terms of susceptibility and conditions for worms to re-establish. That no similar homogenization between different age groups of the host was observed for the re-established population may infer that 'density-dependent limitation' plays a more important role in worm growth compared with factors related to host age. Similar to the results reported by Elkins & Haswell-Elkins (1989), no negative correlation was detected between any worm measurements and the worm burden. However, the patterns displayed in Table 4 suggest that an optimal density (6–10 worms per person) for worm growth may exist. Analysis of the relationship between worm burden and fecundity using data from Bangladesh, Burma, Iran, Madagascar, Mexico and Nigeria showed that, in spite of the geographical variation in female fecundity (measured by EPG per female), the most significant decrease in worm fecundity for most of the countries occurred consistently when the worm burden was between 0 and 5, and that the tendency for fecundity to decrease became moderate when the worm burden was between 5 and 10 (see Fig. 3 in Hall & Holland, 2000). The decrease in egg production may be caused to some extent by an inhibitory process rather than by competition for resources or a 'crowding effect' as pointed by the authors (Hall & Holland, 2000). However, the decrease in egg production may indicate a greater storage of energy in female worms which is beneficial to growth, thus resulting in an increase in size of the worms.

Prevalence and faecal egg counts have been widely used to evaluate and monitor the effectiveness of control programmes. However, in some circumstances caution is warranted in the interpretation of results obtained. As reported previously in the same study area (Peng *et al*. 1996), significant seasonal fluctuations in both prevalence and mean EPG were detected for 6 cross-sectional surveys in local communities within the study year, without any intervention for ascariasis. Furthermore, 1 year after the first universal treatment, both prevalence and EPG were shown to be similar to pre-treatment values (72·1% *vs* 65·6%, 15960 *vs* 14590). Based only on prevalence and EPG data sets, this may lead to a conclusion that a universal treatment regimen would be ineffective, but clearly, this is not supported by the results of the present study based on worm counts.

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