Journal of Helminthology (2004) **78,** 305–310 DOI: 10.1079/JOH2004252

Wet-days: are they better indicators of *Ascaris* infection levels?

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Abstract

Seasonal variation in a particular area may influence the occurrence of helminth infections and determining such fluctuations may help to maximize the beneficial effects of mass treatment. This study determined the seasonal variations in infection levels of *Ascaris lumbricoides* between March 2000 and June 2001 in two selected low-country plantations. Four hundred and seventy seven persons aged between 2 and 74 years (median 13) participated. Stools were tested using the Kato-Katz method and the prevalence and intensity of infection determined. All persons were treated with a single dose of mebendazole. Monthly follow-ups were undertaken with similar stool examinations and treatment given if found positive. Infection and re-infection rates were calculated each month. Rainfall and temperature were recorded each day. Total rainfall, number of wet-days and mean temperature was calculated for each month. The prevalence of *Ascaris* infection was 53.4% and 51.0% at Maliboda and Ayr estates respectively. Highest infection and re-infection rates at Maliboda (37.7%, 37.2%) occurred in June and at Ayr (13.3%, 25.9%) in October 2000 respectively. During the study period, the mean rainfall was 28.1 cm (range 7.4–63.9 cm) and mean temperature 27.6°C (range 22.1°–34.4°C). Significant correlations ($P < 0.05$) were found between the re-infection rate and rainfall, temperature and the number of wet-days. Similar correlations were observed with the infection rate and temperature and the number of wet-days. *Ascaris* infections were found to correlate significantly only with the number of wet-days in a month ($P < 0.01$). Thus, the number of wet-days appears to be a better indicator of *Ascaris* infections than total rainfall or mean temperature.

Introduction

Ascaris lumbricoides is a widespread soil-transmitted nematode causing serious public health problems in developing countries, particularly in tropical regions (Chan, 1997; Crompton, 1999; WHO, 2002). *Ascaris* infection is known to be associated with malnutrition (Crompton, 1986; Stephenson *et al.*, 2000), growth retardation (Stephenson *et al.*, 1989, 1990), reduced learning ability (Nokes *et al.*, 1992), avitaminosis (Tripathy *et al.*, 1972; Sivakumar & Reddy, 1975; Carrera *et al.*, 1984) and even surgical emergencies such as intestinal obstruction on rare occasions (de Silva *et al.*, 1997a).

Ascaris infection is very common in the plantations of Sri Lanka. A survey carried out by Sorensen *et al.* (1996) revealed that 77.0% of children and 69.5% of women of childbearing age harboured *A. lumbricoides* infections. Widespread faecal contamination of the environment occurs due to poor and congested housing conditions and insufficient sanitary facilities. Indiscriminate defaecation on the ground, particularly by children, who do not have or do not use latrines, is a common occurrence (Sorensen *et al.*, 1994).

The prevalence of *Ascaris* infection shows considerable variation in distribution and seasonal occurrence between countries as well as within a country due to the influence of geographical and climatic factors (WHO, 1981). Ascertaining seasonal variation in infection is important as the time frame in which transmission occurs in a specified area can indicate a more appropriate time for the

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https://doi.org/10.1079/JOH2004252 Published online by Cambridge University Press

treatment of the population by mass chemotherapy and also help to predict outbreaks of infection and plan more effective control measures. The present study is the first carried out in Sri Lanka to determine the presence of seasonal variation (with respect to total rainfall, mean temperature and number of wet-days) in the infection and re-infection rates of *A. lumbricoides* in the plantation sector of the country.

Materials and methods

Study area

Two plantations with a high prevalence of infection were selected. The Ayr estate, a rubber plantation, is situated in Padukka (latitude 6°50′N, longitude 80°11′E) at the outskirts of the Colombo district in the Western province of Sri Lanka. The Maliboda estate, a tea and rubber plantation, is situated in Maliboda (latitude 6°54′N, longitude 80°24′E, elevation 274.4 m above sea level) within the Kegalle district of the Sabaragamuwa province of Sri Lanka (fig. 1).

Both plantations were situated in the wet zone of the country averaging more than 4000 mm of rain annually, mostly from the southwest monsoon occurring between May and September (Chandrapala, 1995). The annual average temperature ranged between 25 and 27.5°C with the months of March to May being the warmest (Fernando & Chandrapala, 1995). The inhabitants were estate labourers of similar ethnic and socioeconomic status living in estate lines with minimal latrine facilities.

Collection and examination of stool samples

Four hundred and seventy seven persons aged between 2 and 74 years (median 13) participated in the study. Three hundred and twenty two persons (ages 2 to 50 years, median 11) were from Maliboda and 155 persons (ages 2 to 74 years, median 17) were from Ayr estates. Stool samples were collected from participants each month between February 2000 and June 2001. Ethical clearance for the study was obtained through the Ethical Review Committee of the Faculty of Medicine, University of Colombo.

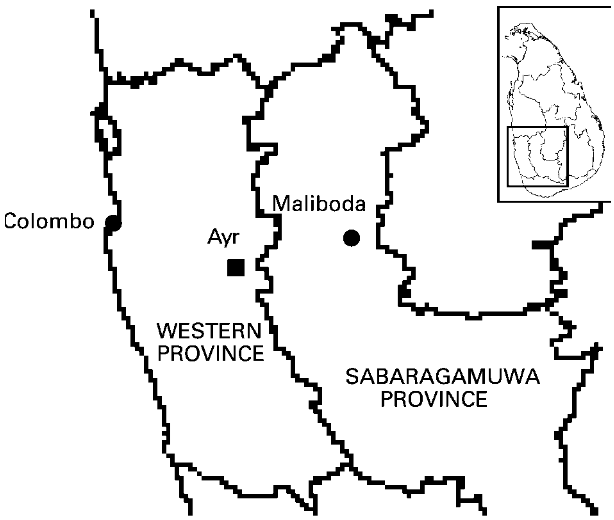

Fig. 1. Map of Sri Lanka (inset) and the locations of the Ayr and Maliboda plantations.

Each stool sample was collected in a clean, wide mouthed, 80 ml plastic container, with an applicator stick. Samples were collected over three consecutive days and transported to the laboratory on each day and maintained at 4°C until examination. All samples were examined either on the same day or on the following day of collection using the Kato-Katz technique (Katz *et al.*, 1972; Suzuki & Sanbe, 1977; WHO, 1991). The prevalence and intensity of soil-transmitted nematode infections were recorded.

Stool surveys

Stool surveys were conducted monthly with 15 surveys being done at Maliboda and 14 at Ayr. The initial pre-treatment stool survey determined the prevalence and intensity of infection in participants from the two estates. A post-treatment stool survey was conducted two weeks later to ensure elimination of worms and follow-up stool surveys were carried out monthly from March 2000 to March 2001 at the Ayr estate and from May 2000 to June 2001 at the Maliboda estate.

Treatment of the study population

All participants were treated with oral mebendazole 500 mg given as a single dose (blanket treatment) following the initial stool survey (pre-treatment survey). Thereafter, all stool smear positives in each monthly survey were treated with a single dose of 500 mg mebendazole.

Infection rates

Infection and re-infection rates were calculated using previously validated methods (Kobayashi, 1983) by determining the percentage of stool egg-positives in the total number of samples examined during each survey, i.e. (number of positives each month / total number of samples examined each month) × 100. Participants were categorized into groups A (egg-negative) and B (egg-positive) according to the results of the pre-treatment stool survey.

The monthly 'infection rate' was calculated as a percentage of individuals who became egg positive from group A [(positives / total examined from group A) × 100]. These positive individuals were thereafter transferred to group B. Similarly, the monthly 're-infection rate' was determined in persons from group B and calculated as they became positive again [(positives / total examined from group B) × 100]. All egg-positive persons were treated and followed up further.

Given the pattern of the life cycle of *Ascaris*, the time of actual transmission was assumed to be three months prior to the date of faecal collection as has been previously described (Kobayashi, 1983; Cabrera, 1984). The infection and re-infection rates were then correlated with total rainfall, the number of wet-days and the mean monthly temperature.

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Rainfall and temperature

Daily rainfall was measured (in cm) every morning using a rain gauge. Minimum and maximum temperatures within a 24-h period were also recorded in degrees Celsius. Total rainfall, the number of wet-days (days with recorded rainfall) and the mean temperature for each month were calculated during the study period.

Statistical analysis

Data from both plantations were combined and analysed using the SPSS statistical software package 11.0 for Windows (SPSS, Chicago, Illinois, USA). Correlations between the infection rates and total rainfall, the mean temperature and the number of wet-days per month were determined using the Spearman's rank correlation test. Multiple regression analyses were further applied to determine the predictive effect of rainfall, temperature and the number of wet-days on the rate of infection, with $P < 0.05$ being considered statistically significant.

Results

General infection patterns

The prevalence of *Ascaris* infections at Maliboda and Ayr estates were 53.4% and 51.0% respectively, and the mean eggs per gram of faeces (MEPG) was 4348.8 (range 0–89,799) at Maliboda and 5178.8 (range 0–53,650) at Ayr, as revealed by the pre-treatment stool surveys. Worm distributions were highly over-dispersed with only 36.7% harbouring moderate to severe infections (>5000 eggs per gram of faeces, WHO, 1987, 2002). The infection and re-infection rates showed similar patterns within as well as between estates (figs 2 and 3). The highest infection and re-infection rates at Maliboda (37.7%, 37.2%) occurred in June and at Ayr (13.3%, 25.9%) in October 2000 respectively.

Climate and infection levels

The mean rainfall precipitation during the study period was 28.1 cm (range 7.4–63.9 cm) with more precipitation at Maliboda (418.6 cm) than at Ayr (340.5 cm). Two peaks of rainfall were observed during the months of May–June and September–October 2000. The mean temperature was 27.6°C (range 22.1°–34.4°C) (figs 2 and 3).

Correlations between total rainfall, the number of wet-days per month and the mean temperature with infection and re-infection rates at the two estates (table 1), revealed statistically significant associations between rainfall, temperature and the number of wet-days and the re-infection rate ($r = 0.506$; $P = 0.010$, $r = -0.471$; $P = 0.017$ and $r = 0.648$; $P = 0.000$ respectively). Only the temperature and number of wet-days per month correlated significantly with infection rate ($r = -0.407$; $P = 0.043$; $r = 0.564$; $P = 0.003$ respectively).

Multiple regression analysis

Multiple regression analysis combining the data from both plantations revealed that, after adjusting for the effect of rainfall and temperature, the number of wet-days played a significant role in the infection levels of *Ascaris* (table 2).

Discussion

Warm and moist environments facilitate the survival of *Ascaris* eggs with moisture making more eggs viable and infective (WHO, 1964; Crompton & Pawlowski, 1985). Rainfall provides the essential moisture for egg survival. An increase in transmission during the rainy months is compatible with this finding. The scattering and sorting action of rain (Brown, 1927; Beaver, 1975; Edirisinghe & Weilgama, 1997) can effectively disperse and transport *Ascaris* eggs over long distances. These eggs can accumulate in shallow pits and ditches which sub-

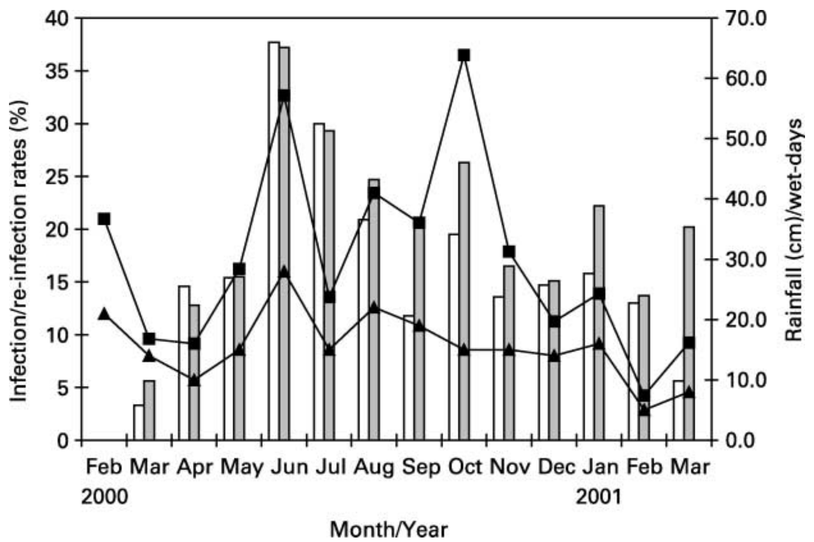

Fig. 2. *Ascaris* infection (□) and re-infection (■) rates correlated with total rainfall (■) and number of wet-days (▲) per month at Maliboda estate during February 2000 to March 2001.

https://doi.org/10.1079/JOH2004252 Published online by Cambridge University Press

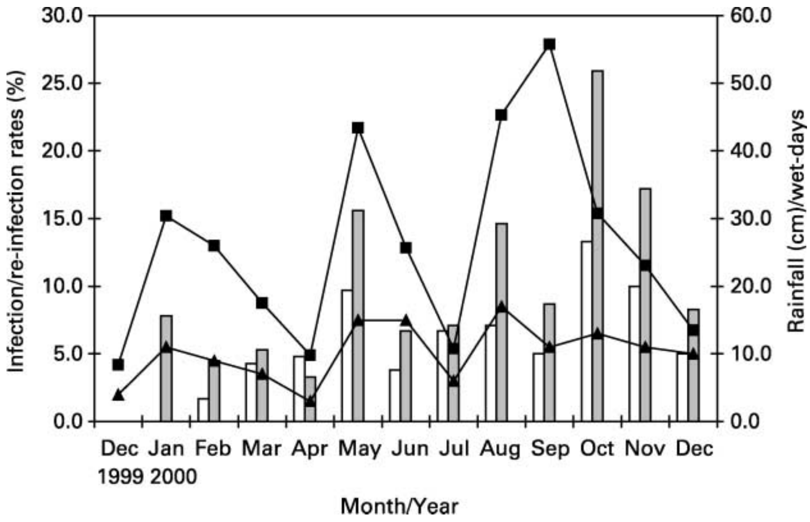

Fig. 3. *Ascaris* infection (□) and re-infection (■) rates correlated with total rainfall (■) and number of wet-days (▲) per month at Ayr estate during December 1999 to December 2000.

sequently serve as natural sand pits for children to play in (Kan, 1985).

Ascaris eggs can also percolate to deeper strata of the soil due to the leaching effects of rain (Brown, 1927; Storey & Phillips, 1985) leaving fewer eggs on the soil surface. The lower rates of transmission during the hot, drier months seen in this study are comparable with the findings of Otto (1929), that eggs exposed to sunlight during the summer became desiccated rapidly and died, depending partly upon the type of soil and humidity. Takasaki (1935) also demonstrated that exposure to direct sunlight (28°–42°C) for 7 or more hours rendered the eggs non-infectious.

In a community where the worm burden is over-dispersed and the majority of human hosts harbour mild infections, it is possible that repeated examinations carried out on different days may reveal low or zero egg counts. However, in the present study, all participants were given treatment (blanket treatment) following the initial stool survey. Those found to be stool egg-negative were also treated because they may harbour sexually immature worms or low egg counts (i.e. giving false negative results). Thereafter any individual found to be egg-positive during each follow-up survey was again treated. Mebendazole is known to be highly effective against *Ascaris* infections giving cure rates of 90–100% (de Silva *et al.*, 1997b). Thus, it was assumed that the egg-positives encountered from each follow-up survey were new infections.

This study has clearly shown that the number of wet-days per month plays a more significant role in the transmission of *Ascaris* infection than the total amount of rainfall per month. A high total rainfall could occur due to steady rain throughout the month or due to sporadic heavy showers. Heavy rainfall is more likely to disperse the eggs horizontally and vertically. A steady rainfall throughout the month would help maintain the required moisture, making more *Ascaris* eggs viable and infective.

The correlation between rainfall and transmission of soil-transmitted nematodes has been found to differ in various countries where this phenomenon has been studied. Higher transmission rates were observed during the drier months in Japan (Pan *et al.*, 1953) and in the Philippines (Cabrera, 1984), in contrast to studies in Nepal (Gurubacharya, 1986) and Bangladesh (Rahman, 1986), although the number of wet-days per month were not considered in these studies.

The present study, which is the first to analyse the effect of seasonal variation on the incidence of *Ascaris* infection in the plantation sector of Sri Lanka, clearly demonstrates

Table 1. Spearman's rank correlation between total rainfall, mean temperature, number of wet-days and the *Ascaris* infection and re-infection rates at Maliboda and Ayr plantations from December 1999 to March 2001.

	Infection rate		Re-infection rate	
	r-value	*P*-value	r-value	*P*-value
Rainfall	0.308	0.134	0.506	**0.010**
Temperature	−0.407	**0.043**	−0.471	**0.017**
Wet-days	0.564	**0.003**	0.648	**0.000**

Statistically significant values are indicated in bold type.

Table 2. Summary of multiple regression analyses between total rainfall, mean temperature, number of wet-days and the *Ascaris* infection and re-infection rates at Maliboda and Ayr plantations from December 1999 to March 2001.

Dependent variable	Independent variable	Regression coefficient	*P*-value
Infection rate	Rainfall	−0.068	0.596
	Temperature	−0.548	0.780
	Wet-days	1.155	**0.008**
Re-infection rate	Rainfall	−0.026	0.831
	Temperature	−0.999	0.591
	Wet-days	1.001	**0.013**

Statistically significant values are indicated in bold type.

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the positive correlation existing between the numbers of wet-days per month and the incidence of *Ascaris* infections. This enables a more appropriate and effective time for scheduling mass chemotherapy of the population, in addition to predicting outbreaks of infection and planning more effective control measures. It remains to be determined whether this correlation exists in the other parts of the country and in other countries as well.

Acknowledgements

We sincerely thank the University of Colombo and the Japan Association of Parasite Control for providing financial support for the study. We also thank Dr Indira Hettiarachchi, Director, Health and Women's Affairs Division of the Plantation Housing and Social Welfare Trust and her staff for the cooperation extended to facilitate working in the plantations. We are grateful to Dr Rajitha Wickremasinghe for advice on statistical analysis and Mrs Himali Gunathilake for technical support.

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(Accepted 20 May 2004)

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