

Hookworm infection : Prevalence among the tribal populations of Meghalaya (Northeast India) and development of the infective stage larvae under varied environmental conditions*

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Abstract

The prevalence of hookworm infection was ascertained among the tribal populations, the Khasis, the Jaintias and the Garos of Nongkya, Sutnga and Williamnagar, respectively at three different periods of the year, i.e., pre-monsoon (March-June), monsoon (July-October) and post-monsoon (November-February) that relate to different climatic conditions prevailing through the year. All the positive samples were cultured for larvae, which in all the cases were identified as belonging to *Necator americanus*. Females showed a higher prevalence infection in most of the cases. Though no age-related trend in prevalence was evident, the intensity of infection was found to be more in the higher age groups i.e., 15-19 years and 20 years and above. The infected individuals in all the three study sites were treated with mebendazole in the post-monsoon time. Three weeks post treatment, the infection rate among them lowered down but showed an increasing trend again after 5-7 months and beyond. Among the environmental objects, soil was found to be the important transmitting medium. The optimal periods of development and hatching of eggs and development of L₃ larvae coincided with warmer temperatures and higher humidity, which also favoured a longer period of survival of L₃ larvae in the soil.

(Keywords : hookworm/*Necator americanus*/tribal population)

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Hookworm infection is widely prevalent infecting over a billion people worldwide particularly in the tropical and sub-tropical countries¹. The infection is implicated in the etiology of iron-deficiency anemia that causes chronic intestinal blood loss², and as estimated 50,000 deaths per year occur worldwide as a result of hookworm infection alone³. Various other nutritional deficiencies including protein, folic acid and Vitamin B₁₂ have been reported in individuals with hookworm infections⁴. Heavy hookworm infections may also lead to retarded growth and development of children⁵. Hookworm thresholds are based on upper and lower bound estimates of the relationship between infection intensity and anemia⁶. As per estimates, the highest prevalence morbidity related to hookworm infection is in the Indian sub-continent⁷. The prevalence and severity of hookworm infection vary widely in different parts of the globe because of the factors such as climatic, socio-economic, educational and environmental sanitary conditions and to a great extent also show relationship with age and sex of the host⁸.

While *Ancylostoma duodenale* is usually found in cooler drier regions in Europe, the Middle East, the Mediterra-

nean, North Africa, Pakistan and Northern India, *Necator americanus* predominates in the Americas, Central Africa, Eastern and Southern India, Indonesia and South Pacific⁹.

In context of India, extensive investigation carried out on similar lines covering almost all provinces of erstwhile British India, provided immense information on the prevalence and epidemiology of hookworm infection in relation to the prevailing geographical, climatic and socio-cultural conditions¹⁰. Recent studies in Assam, carried out after a gap of nearly 8 decades, still show a high prevalence of hookworm infection in human populations particularly those in the tea gardens¹¹. However, information regarding the species of hookworm implicated in infection in North-east Indian is lacking. This information is needed to assess the clinical significance of the hookworm infection, since blood loss due to the species commonly infecting man varies¹². In the state of Meghalaya, which represents varied climates viz., hot mild, sub-tropical and humid, reports from the various medical agencies suggested a considerable prevalence of hookworm infection in the region.

Many reports deal with the investigations into the ways in which nematode infections thrive in society and in many of these, soil and field-grown vegetables, in particular, have been found to be the major transmitting media for nematode infection in the area¹³. Transmission of the geo-helminthic parasites has a link to topography-related climatic variables to differing extents since their life cycle includes stages outside the host with the

environment. Temperature, moisture and day light are important limiting factors that control the hatching of the eggs and development of L₃ infective larvae of gastrointestinal helminths¹⁴. Knowledge of the geographic distribution and seasonal occurrence of these parasites is of practical importance when devising control programs for a particular region. The present study (1996-1999) was undertaken to assess the status of hookworm infection in some chosen areas of Meghalaya.

Study area :

Nongkya : Located in Umsning (Ri-Bhoi District) about 33 kms north of the state capital Shillong, the area represents a warm and humid climate experiencing rainfall almost throughout the year, with high relative humidity except for a short period of dry season usually during winter (November-February),. (Table 1). This village comprised of the Khasi tribal population only with a total of 489 individuals. The eco-system basis of the area is paddy field and the occupation, agriculture. The level of hygiene and sanitation is by and large very poor. Only 2% of the households have latrines and all the inhabitants use paper for self-ablution as a common practice.

Sutnga : This area is located in the Jaintia Hills District about 30 kms from Jowai, the district headquarter and about 90 km south of the state capital Shillong. This village represents a warm but very less humid climate compared to Nongkya village. It too experiences rainfall throughout the year except for a short period of dry season (November-February). About 80% of the area is covered with coalfields. Hence the villagers are either

directly or indirectly involved in coal mining. Their living condition is very poor with only 60 % having proper latrines. The village had a population of 804 individuals belonging to the Jaintia tribe only.

Williamnagar : This is the head quarter of the East Garo Hills District and is

Table 1-Mean temperature (°C) and relative humidity (RH-%) recorded in the three study sites during the study period.

Study sites	Nongkya		Sutnga			Williamnagar			
	Temperature		RH	Temperature		RH	Temperature		
	Min.	Max.		Min.	Max.		Min.	Max.	
Pre-monsoon (Nov-Feb.)	7.5	19		6	14		9	20.5	
Monsoon (March-Jun)	13.5	37	67-90	10.5	24.5	50-80	18	42	68-9
Post-monsoon (July-Oct.)	15.5	37		10.5	25		15	32	

situated about 330 km west of the state capital Shillong. The climate is hot and humid. A few inhabitants have simple pit latrine. The villagers are engaged in poultry, piggery, cattle farming and paddy cultivation for their livelihood. The place had a population of 1142, of which 962 belonged to the Garo tribe.

Survey and analysis :

Faecal samples were examined for hookworm and other intestinal helminthes, if any, using Kato-katz thick smear¹⁵ and direct faecal smear methods. To determine the hookworm species, positive hookworm samples were cultured using the modified Harada-Mori filter-paper-strip cultivation method¹⁶. The intensity of infection was assessed by counting the number of eggs per gram (EPG) of faecal sample, following Stoll's dilution counting method¹⁷ and infections were classified according to individual egg counts as heavy, moderate,

or light, following WHO recommendations¹⁸.

The species identification was determined on the basis of the subtle morphological characteristics of the L₃ larvae and the adult worm¹⁹.

Immediately after the post-monsoon survey the infected individuals were treated with a single dose of mebendazole (500 mg) under the supervision of the medical practitioner of the respective village. Stool examination was carried out again and the prevalence recorded at time periods of 3 weeks, 5-7 months and 9-12 months post treatment in all the three study sites. Data was also collected on the living conditions of the infected individuals.

Environmental objects such as soil, water and vegetables were collected from the three study sites and examined for positivity of hookworm eggs or larvae following Baermann's and Salt floatation techniques^{20,21}.

Freshly passed out hookworm eggs from faeces of infected subjects were collected and cultured, following Charcoal culture technique²² in a BOD incubator at various temperatures (between 2 and 35°C) with RH of 55-60, 65-70 and 80-85%

(corresponding to the ambient climatic conditions that prevail in winter and spring (i.e., late November-March), autumn (September-early November) and summer (April-August), respectively. Three replicates of each culture were prepared using sample from a single infected subject.

Based on the morphological characters of both the larvae retrieved from culture and the adult worms, the hookworm species prevailing in all the studied sites were identified as *Necator americanus*.

Prevalence of hookworm infection :

In Nongkya, of the 425 subjects examined, 58.57%, 68.94% and 74.10% were found infected with single or multiple species infections involving hookworm (H), *Ascaris* (A) and *Trichuris* (T) in the pre-monsoon and post-monsoon periods of the year respectively. The prevalence of hookworm infection alone was 7.29-9.17% all through the year. Dualfection (H+A) and triplefection, (H+A+T) were also recorded (Table 2). In Sutnga, of the 700 subjects examined, 90.71%, 93.28% and 97.85% were found infected with single or multiple

species infection in the three different periods, respectively. Though dualfection with H+A, H+T and A+T and triplefection (H+A+T) were also found, the single species infection of hookworm showed a high prevalence (Table 3). In Williamnagar, of the 962 subjects examined, 58.93%, 81.49% and 91.58% were found infected with single or multiple species infection in the three different periods respectively. The prevalence of hookworm infection alone ranged between 25.36-46.86% (Table 4).

In Nongkya the age group 5-9 years was identified as the high-risk age group, while in Sutnga and Williamnagar it was 10-14 years for hookworm infection. In most cases, females were more infected than males in all the three village areas, though the differences in prevalence and intensity were not significant.

Of the environmental objects only the soil samples were found to show contamination with hookworm eggs/larvae from all the three study sites.

Table 2-Prevalence of hookworm and its associated gastrointestinal nematode infection in Nongkya village : No. of subjects examined = 425, M = 219, F = 206.

Infection	Pre-monsoon		Monsoon		Post-monsoon	
	No. infected	Prevalence %	No. infected	Prevalence %	No. infected	Prevalence %
Hookworm	31	7.29	38	8.94	39	9.17
<i>Ascaris</i>	162	38.11	184	43.29	186	43.76
<i>Trichuris</i>	4	0.94	4	0.94	4	0.94
H+A	44	10.35	57	13.41	68	16
H+T	-	-	-	-	-	-
A+T	-	-	-	-	-	-
H+A+T	8	1.88	10	2.35	18	4.23
Total	249	58.57	293	68.94	315	74.11

Table 3—Prevalence of Hookworm and its associated gastrointestinal nematode infection in Sutnga village : No. of subjects examined = 700, M = 410, F = 290.

Infection	Pre-monsoon		Monsoon		Post-monsoon	
	No. infected	Prevalence %	No. infected	Prevalence %	No. infected	Prevalence %
Hookworm	267	38.14	279	39.85	281	40.14
<i>Ascaris</i>	309	44.42	328	46.00	344	49.00
<i>Trichuris</i>	-	-	-	-	1	0.14
H+A	45	6.42	46	6.57	48	6.85
T	-	-	-	-	2	0.28
A+T	7	1.00	3	0.42	5	0.71
H+A+T	5	0.71	3	0.42	5	0.71
Total	635	90.71	653	93.28	685	97.85

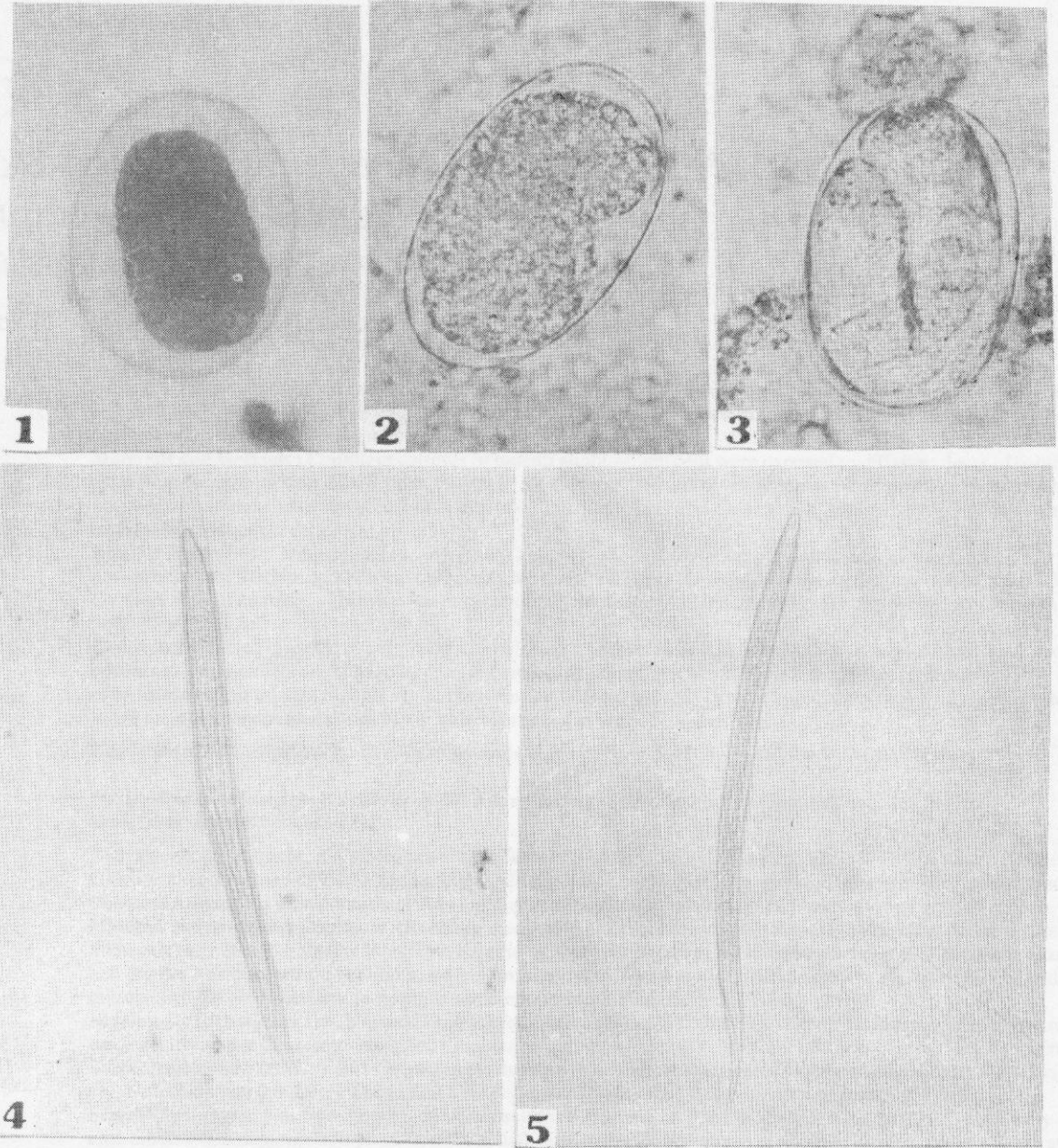
Table 4—Prevalence of Hookworm and its associated gastro-intestinal nematode infection in Williamnagar : No. of subjects examined = 962, M = 448, F = 514.

Infection	Pre-monsoon		Monsoon		Post-monsoon	
	No. infected	Prevalence %	No. infected	Prevalence %	No. infected	Prevalence %
Hookworm	244	25.36	407	42.27	451	46.86
<i>Ascaris</i>	301	31.28	351	36.48	385	40.02
<i>Trichuris</i>	-	-	2	0.20	2	0.20
H+A	19	1.97	21	2.18	38	3.95
H+T	-	-	-	-	2	0.20
A+T	2	0.20	2	0.20	2	0.20
H+A+T	1	0.10	1	0.10	1	0.10
Total	567	58.93	784	81.49	881	91.58

After treatment the prevalence of infection was found to be low in all the three study areas. The number of subjects infected with single or multiple species infection declined to 6.82%, 8.14% and 9.35%, 3 weeks post treatment, but it rose again (24.7, 18 and 17.87%, respectively) after 5-7 months and further beyond, following treatment.

No development of eggs incubated at 14°C was observed. The rate of

development and hatching of hookworm eggs was observed to be low at 14°C , RH 57.5% and highest at 28°C , RH 82.5%. It took only about 8-10 days for the eggs to develop and hatch at $14-18^{\circ}\text{C}$, while only 2-3 days at $22-28^{\circ}\text{C}$ at the same RH of 82.5%. Beyond 28°C a decline both in development and hatching rate was observed. The various developmental stages of the egg and larvae post hatching at optimal temperature (28°C) an RH (82.5%) are shown in Figs. 1-5.



Figs. 1-5. Development of *Necator americanus*

1. Fertilised egg (embryonation stage)
2. Morula stage
3. Infective egg containing larval stage
4. Rhabditiform larva stage
5. Filariform larva

The L₃ filariform stage of larvae was observed in the culture between 8-9 days post hatching at 22-25°C, RH 82.5%. However, the time period of development to L₃ stage was prolonged to 9-12 days in the culture set at lower humidity. The rate of turnover of L₃ and their survival rate showed a rise with increase temperature to 28°C, but beyond which showed a decline. The developed L₃ larvae were observed to survive for longer time (till about 80 days) at 28°C, while only about 55 days at 14°C. At very low temperature (2-4°C) the filariform larvae did not survive beyond 4 days.

Discussion :

Necator americanus was found to be the only hookworm species infecting human subjects in the three study areas. Nawalinski²³ also reported *N. americanus* as a significant proportion of hookworm infection in rural communities in India.

The higher prevalence of hookworm infection in the present study in females than in males in most of the cases may be related to the profession of the subjects; as per the social set up of the natives of Meghalaya, the women folk work more in the fields and outdoors, are therefore prone to more infection as compared to the males. Gandhi²⁴ also reported that females were more likely to have moderate or heavy infections, whereas the males were more likely to have light infections and that the infected subjects harboured a combination of geohelminthic nematodes. Intestinal parasitoses involving multiple species infections are of common occurrence in South-east Asia^{8,25,26}. The present study identified the high-risk age group for the hookworm infection as 5-9 years in Nongkya, which represents a typical rural

position, and 10-14 years in Sutnga and Williamnagar, the latter two places relate to semi urban epidemiology as they include coal miners and animal farmers²⁷. The prevalence thus seems to be related to the status of personal hygiene practised in the said age groups and also more frequent exposure to the places such as house yards, school compounds, playgrounds and public places which are contaminated with hookworm eggs or larvae. Toma²⁸ identified the age group 4-14 years to be most likely infected with hookworm. However in contrast to the present finding, other studies suggest a different pattern in which both prevalence and intensity of infection showed a rise with age²⁹ and the worms tended to be more aggregated among adults^{24,26}.

All the positive cases of hookworm infection showed light infection. This low intensity of infection in the subjects may be due to inter-species competition. The estimation of worm burden which is based on EPG count may have several sources of variation which can make the inference of intensity status for individuals imprecise²⁹, egg counts may also vary from day to day within individuals and the fecundity of female worms is also dependent on worm density³⁰. However even with mild to moderate worm burden, the continued presence of worms in the marginally nourished subjects could be a cause of concern in contributing to anaemia conditions³¹.

The investigation showed that soil in places of frequent human exposure in the study area is a potential source of hookworm infection to the inhabitants. Geohelminthic intestinal parasites have been repeatedly reported as common infections in human population of several

regions of India³². Due to the enormous number of eggs produced by one adult female worm, a single contaminated stool passed in the soil is sufficient to infect an entire population.

After treatment with single dose of mebendazole, the prevalence of infection initially declined, but showed a rise after a lapse of several months perhaps because of endemicity of environment with infective stages. Chemotherapy, thus providing a periodic cure, did not check re-infection of predisposition to infection³³.

Temperature and humidity have a marked effect on the development and hatching of hookworm eggs. Of the two factors, temperature played an important role, since at low temperatures <14°C (corresponding to the cold winter temperatures of late November to early February) no development of eggs occurred even when the RH decreased or increased. At higher temperature (30°C) the eggs took much less time in hatching, while the rate of hatching, survival of L₃ as well as their longevity declined compared to those incubated at 20°C. The optimal development temperature does not necessarily relate to larvae with highest larval longevity³⁴.

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