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Hookworm infection among the rural tribal populations of Meghalaya (North-east India)

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The prevalence of hookworm infection was ascertained in the rural areas of Nongkya, Sutnga and Williamnagar that are represented by three tribal populations, the Khasis, the Jaintias and the Garos, respectively at 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. While the inhabitants of Nongkya and Williamnagar mainly practise agriculture, those of Sutnga are involved in coal mining. Direct and Kato-Katz thick smear methods were used for stool examination. Using a modified Harada-Mori filter paper strip culture method, all the positive samples were cultured for larvae, which in all the cases were identified as *Necator americanus*. The prevalence of hookworm infection in Nongkya was 7.29%, 8.94% and 9.17%, in Sutnga it was 38.14%, 39.85% and 40.14%, while in Williamnagar it was 25.36%, 42.27% and 46.86%, respectively during the three periods. Dual and triple infections involving *Ascaris* and *Trichuris* were also recorded, though of much lower prevalence than that of single-species infection of hookworm in all the study sites. The prevalence was highest in the 5-9 years age group in Nongkya during the monsoon period that coincides with the paddy harvesting time while the 10-14 years age group showed the highest prevalence in Sutnga and in Williamnagar in the post-monsoon period. No age-related trends of prevalence and intensity of infection were evident. Females showed a higher prevalence than males in most of the cases.

The infected individuals were treated with mebendazole just after the post-monsoon period. Three weeks post-treatment the infection rate among them lowered down but again showed an upsurge after 5-7 months and continued to be the same or at a slightly higher level after 9-12 months. It appears that the infected individuals have a predisposition to hookworm infection, not by chance but due to behavioural and socio-economic factors.

Key Words: Hookworm infection, Human population, Meghalaya, Northeast India

Hookworm infection is widely prevalent infecting over a billion people worldwide particularly in the tropical and sub-tropical countries with prevalence as high as 95% in certain communities of developing countries (Hoagland and Schad, 1978; Walsh and Warren, 1979). These infections are implicated in the etiology of iron-deficiency anemia that causes chronic intestinal blood loss (Roche and Layrisse, 1966; Woodruff, 1982), and as estimated 50,000 deaths per year occur worldwide as a result of hookworm infection alone (Walden, 1991). Various other nutritional deficiencies including protein, folic acid and Vitamin B_{12} have been reported in individuals with hookworm infections (Li, 1990). Besides causing iron deficiency anemia in populations with poor diets (Stoltfuzs et al., 1997) heavy hookworm infections may also lead to retarded growth and development of children (Hotez and Pritchard, 1995). It has also been cited as a major concern for women's health because of its potential impact on maternal anemia and reproductive outcomes (WHO, 1994). Hookworm thresholds are based on upper and lower bound estimates of the relationship between infection intensity and anemia (Lwambo et al., 1992). Some analysis has provided estimates of the number of women that are both pregnant and infected with hookworm at a worm burden likely to be associated with disease (Navitsky et al., 1998). As per estimates, the highest morbidity related to hookworm infection is

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in the Indian sub-continent (Chan et al., 1994; Weidong et al., 1995).

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 host (Kan, 1989; Bakta et al., 1993).

Hookworms mainly comprise two species viz., *Necator americanus* and *Ancylostoma duodenale,* which occur commonly in the tropical and sub-tropical regions. Of these, *A. duodenale* has been reported to be responsible for most of the infections usually more abundant in rural as opposed to urban communities (Gilles, 1985; WHO, 1994). While A. *duodenale* is usually found in cooler drier regions in Europe, the Middle East, the Mediterranean, North Africa, Pakistan and Northern India, *N. americanus* predominates in the Americas, Central Africa, Eastern and Southern India, Indonesia and South Pacific (De Cameri, 1974; Schad and Warren, 1990).

In context of India, extensive investigation carried out on similar lines by Chandler (1926-1928) and 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 conditions. Chandler (1925) reported that while the entire northwestern and Deccan part of India was entirely free from hookworm infection, a high incidence of it occurred in Bihar, Orissa and some parts of the (erstwhile) Assam state. 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 gardens (RMRC, 1993). However, information regarding the species of hookworm implicated in infection in North-east India 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 varies (Gilles et al, 1964; WHO, 1981). In the state of Meghalaya, which represents varied climates viz., hot, mild, sub-tropical and humid, reports from the various medical agencies suggest a considerable prevalence of hookworm infection in the region. The present study was, therefore, undertaken with a view to investigating the status of hookworm infection in human population of Meghalaya and assessing some epidemiological factors on prevalence of the infection.

MATERIALS AND METHODS

The study area consisted of three villages, Nongkya, Sutnga and Williamnagar comprising of three tribal populations of the state.

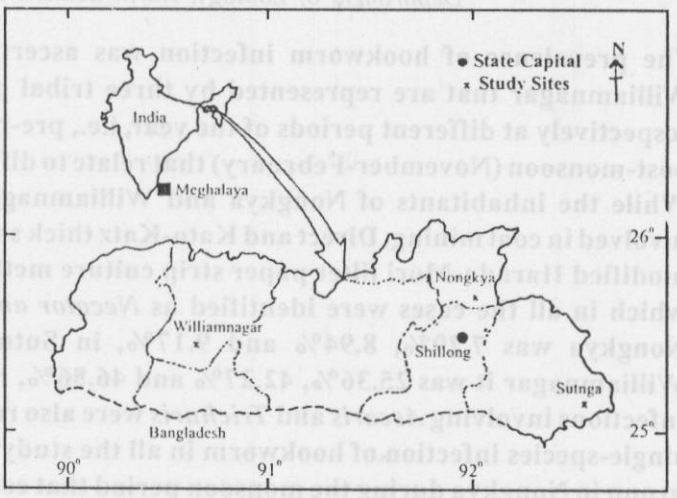

Fig. 1. Map of Meghalaya showing the study sites.

Study area (Fig 1)

1. Nongkya: This village is located in Umsning which falls in the Ri-Bhoi District located about 33 km north of the state capital Shillong. The area represents a warm and humid climate experiencing rainfall almost throughout the year, which results in high relative humidity except for a short period of dry season usually during winter (November-February) when the rainfall is very less. The mean minimum and maximum temperatures in the area during the period of study (1996-1999) were 7.5°C and 19°C, respectively during the dry winter months (November-February), 13.5°C and 37°C during the pre-monsoon period (March-June) and 15.5°C and 35°C during the monsoon period (July-October), while the mean daily relative humidity ranged between 67-90% during the year 1996-99 (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. Most of the children go to school up to primary standard, while the adults are generally uneducated. The level of hygiene and sanitation is by and large very poor. People mostly

Table 1. Mean temperature (°C) and relative humidity (RH- %) recorded in the three study sites during the study period (1996-1999)

	Nongkya			Sutnga			Williamnagar		
	Temperature		RH	Temperature		RH	Temperature		RH
Period	Min	Max		Min	Max		Min	Max	
Pre-monsoon (Nov - Feb)	7.5	19.0		6.0	14.0		9.0	25.5	
Monsoon (March - June)	13.5	37.0	67-90	10.5	27.5	50-80	18.0	42.0	68-90
Post-monsoon (July - Oct)	15.5	35.0		10.5	25.0		15.0	32.0	

defecate in the open fields and behind their house yards. Only 2% of the households have latrines and all the inhabitants use paper for self-ablution as a common practice. Few pigs are reared and a number of dogs are domesticated. There being no water supply to houses, water is collected from streams near by or from a common tap which provides water only once a day. Most of the houses have thatched roofs.

2. Sutnga: This area is located in the Jaintia Hills District about 30 km 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) when there is very less rainfall. The mean minimum and maximum monthly temperatures during the study period were 6°C and 14°C during the dry winter months (November-February), 10.5°C and 27.5°C during the pre-monsoon period (March-June) and 10.5°C and 25°C during the monsoon period (July-October). The mean daily relative humidity ranged between 50-80%. About 80% of the area is covered with coalfields and even most of the houses are built on top of the coalmines. Hence the villagers are either directly or indirectly involved in coal mining. Though the people are well to do, yet the majority are illiterate. Only 10% of the children go to school, and of the adults only 5% are educated. Their living condition is very poor with only 60% having proper latrines, while the rest defecate in near by bushes and use paper for self-ablution as a common practice. As the soil is not suitable for any cultivation, the inhabitants depend entirely on the weekly local market for their food supply. Water supply to houses is not available. A common community water tap or stored rainwater or open wells are the source of water. The houses are made of polyvinyl sheets and wood pieces and a few are thatched huts. The village had a population of 804 individuals belonging to the Jaintia tribe only.

3. Williamnagar: This is the headquarter of the East Garo Hills District and is situated about 330 km west of the state capital Shillong. The climate is hot and humid, the mean daily relative humidity being 68-90%. The mean minimum and maximum temperatures during the period of the study were 9°C and 20.5°C during the post-monsoon period (November-February), but during the pre-monsoon (March-June) and monsoon period (July-October), the temperatures recorded were 18°C and 42°C, and 15°C and 32°C, respectively. Most of the inhabitants reside in temporary huts, few have simple pit latrine and the others defecate in near by bushes or near by streams or rivers and use water for self-ablution. The house water supply is not available, hence most of them collect water from near by streams or store rainwater. 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

Village programmes were initiated with a discussion with village headmen of the three study areas to explain the purpose of the work and pictures of helminth parasites and photographs of infected humans were shown to the villagers. Sterilized plastic bottles were given for sample collection to participating individuals and their names, age, sex, occupation and socio-economic status, family

relations and house locations were recorded. The next day fecal samples were collected from the inhabitants who voluntarily agreed to have their stool examined.

Samples were examined for eggs of hookworm and other intestinal helminths, if any, using Kato-Katz thick smear (Martin and Beaver, 1968) and direct faecal smear method (Beaver, 1950). To determine the hookworm species, positive hookworm samples were cultured using the modified Harada-Mori filter paper-strip cultivation method (Kosin et al., 1973). The intensity of infection was assessed by counting the number of eggs per gram (EPG) of faecal sample, following Stoll's dilution counting method (WHO, 1963) and infections were classified according to individual egg counts as heavy, moderate, or light, following WHO recommendations (Renganathan et al., 1995).

The filariform larvae were collected and mounted in lacto-phenol with cotton blue. Adult worms whenever recovered from stool samples were processed for final mounting in glycerine jelly. The species identification was determined on the basis of the subtle morphological characteristics of the L3 larvae and the adult worm (Pawlowski et al., 1991).

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.

RESULTS

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 74.11 % were found infected with single or multiple species infections involving hookworm (H), *Ascaris* (A) and *Trichuris* (T) in the pre-monsoon, 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. 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 (38.14-40.14%). 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. H+A, H+T and A+T were found as dualfection and H+A+T, as triplefection. The prevalence of hookworm infection alone ranged between 25.36-46.86% (Table II).

In the present study in Nongkya the age group 5-9 years was identified as the high-risk age group with 2.82%, 4% and 4% prevalence in the three periods of examination, respectively, while in Sutnga and Williamnagar the age group 10-14 was identified as the high-risk age group for hookworm infection (Table III). 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 (Table IV).

The living conditions of the infected individuals were also by and large very poor. From the data collected it was revealed that the prevalence of infection was more among those with no sanitary facilities and water supply (Table V).

After treatment the prevalence of infection was found to be low in all the three study areas. In Nongkya, the number of subjects infected with single or multiple species infection declined to 6.82% 3 weeks post treatment, but their number again rose to 24.7% after 5-7 months and 28.47% after 9-12 months following treatment. In Sutnga, the hookworm infection declined to 8.14% after 3 weeks post treatment but after 5-7 months and 9-12 months the infection rate increased to 18% and 26.57%, respectively. Similarly in Williamnagar, the infection rate declined to 9.35% but increased again after 5-7 months (17.87%) and further increased after 9-12 months (23.90%) (Table VI).

DISCUSSION

Necator americanus was found to be the only

Table II. Prevalence of hookworm and associated gastrointestinal nematode infections in Meghalaya

	Pre-monsoon		Monsoon		Post-monsoon	
	No. infected	Prevalence %	No. infected	Prevalence %	No. infected	Prevalence %
Nongkya						
(No. of subjects examined = 425)						
Hookworm	31	7.29	38	8.94	39	9.17
Ascaris	162	23.11	184	43.29	186	43.76
Trichuris	4	0.94	4	0.94	4	0.94
H+A	44	10.35	57	13.41	68	16.00
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
Sutnga						
(No. of subjects examined = 700)						
Hookworm	267	38.14	279	39.85	281	40.14
Ascaris	309	44.42	328	46.00	344	49.00
Trichuris	-	-	-	-	1	0.14
H+A	45	6.42	46	6.57	48	6.85
H+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	83.28	685	97.85
Williamnagar						
(No.of subjects examined = 962)						
Hookworm	244	25.36	407	42.27	451	46.86
Ascaris	301	31.28	351	36.48	385	40.02
Trichuris	-	-	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	1	0.10	1	0.10
H+A+T	1	0.10	1	0.10	1	0.10
Total	567	58.93	784	81.49	881	91.58

Table III. Age-wise prevalence and intensity of hookworm infection in Meghalaya

Age group (Yrs.)	No. of subject	Prevalence%			Mean EPG ± SD		
		PrM	M	PM	PrM	M	PM
Nongkya							
0-4	86	0.70	0.70	0.70	600.00±57.15	645.32±62.50	647.30±63.50
5-9	86	2.82	4.00	4.00	780.80±212.30	789.19±214.60	790.50±215.50
10-14	67	1.64	2.35	2.58	742.80±129.40	764.84±145.50	796.50±150.80
15-19	53	1.17	1.41	1.41	660.00±159.40	700.00±115.80	795.50±147.50
20+	133	0.94	0.94	0.94	457.50±184.40	665.45±195.90	796.50±210.00
Sutnga							
0-4	79	3.42	1.85	2.85	550.55±64.20	680.50±68.30	685.50±68.28
5-9	155	6.00	5.42	3.57	700.05±154.80	764.85±160.54	765.50±161.15
10-14	212	11.71	13.14	15.14	700.52±154.95	750.90±158.65	960.04±176.35
15-19	128	10.85	11.14	11.14	700.68±154.92	1200.55±214.38	1540.58±210.14
20+	126	6.14	8.28	9.42	680.55±144.44	1558.45±224.26	1750.05±246.39
Williamnagar							
0-4	98	1.87	2.80	2.80	555.55±92.56	640.05±94.25	650.78±98.11
5-9	270	6.02	10.08	11.53	684.84±98.45	756.52±104.09	760.56±108.89
10-14	279	7.58	12.37	15.28	844.29±113.67	890.56±116.67	910.75±124.45
15-19	197	5.50	8.62	8.93	894.21±119.56	1110.45±144.70	1250.00±147.09
20+	118	4.36	8.41	8.31	974.40±126.35	1542.78±157.78	1584.45±156.09

PrM - pre-monsoon, M - monsoon, PM - post-monsoon, EPG - eggs per gram of faeces.

Table IV. Sex-wise prevalence and intensity of hookworm infection in the three study areas

Study period	Pre-monsoon		Monsoon		Post-monsoon	
Study areas	Prev. % (M/F)	Mean EPG (M/F±SD)	Prev. % (M/F)	Mean EPG (M/F±SD)	Prev. % (M/F)	Mean EPG (M/F±SD)
Nongkya (M=219, F=206)	6.39/ 8.25	712.57±236.60/ 678.23±177.10	7.30/ 10.67	752.84±238.32/ 767.05±239.97	9.13 9.22	918.18±312.04/ 974.23±345.06
Sutnga (M=410, F=290)	27.07/ 8.25	749.99±235.13/ 689.02±247.04	38.00/ 42.41	1249.23±298.17/ 1449.04±301.06	45.36/ 32.75	1763.00±499.18/ 1783.21±499.92
Williamnagar (M=448, F=514)	29.46/ 21.78	845.56±296.18/ 836.19±295.54	39.28/ 44.94	1605.24±384.26/ 1600.23±384.08	52.23/ 42.21	2018.62±545.12/ 2114.32±568.08

EPG - egg per gram of faeces, M - male, F- female, Prev. - prevalence.

Table V. Living conditions and personal habits of hookworm - infected subjects in the tree study areas of Meghalaya (n = number of subjects)

Study areas/ Time period	Nongkya			Sutnga			Williamnagar		
	PrM	M	PM	PrM	M	PM	PrM	M	PM
Living conditions	n=31	n=38	n=39	n=267	n=279	n=281	n=244	n=407	n=451
Latrine									
a. Own	22.58	21.05	17.94	24.55	25.69	25.41	26.22	23.45	24.5
b. Public places	77.41	78.94	82.05	75.44	74.30	74.58	73.77	76.54	75.49
Water supply									
a. Common tap	38.70	39.47	46.15	40.11	46.36	48.12	0.00	0.00	0.00
b. Streams/rivers	61.29	60.52	53.84	59.88	58.63	51.87	100	100	100
Self - ablution									
a. Water	-	-	-	-	-	-	100	100	100
b. Paper	100	100	100	100	100	100	-	-	-
Occupation									
a. Agriculture	64.50	60.52	69.23	-	-	-	22.95	13.68	14.52
b. Animal farming	19.35	26.31	15.38	-	-	-	63.93	74.26	74.64
c. Coal mining	-	-	-	60.05	60.47	72.92	-	-	-
d. Others	16.12	13.15	15.28	39.19	39.52	27.07	36.18	25.55	25.29

PrM = Pre monsoon, M = Monsoon, PM = Post monsoon.

hookworm species infecting human subjects in the three study areas. Nawalinski et al. (1978) also reported *N. americanus* as a significant proportion of hookworm infection in rural communities in India. Lili et al. (2000) identified *N. americanus* as the exclusive hookworm in Lushui Puer counties in Yunnan Province in China. Similarly, Gandhi et al. (2001) determined *N. americanus* as the predominant species of hookworm infection in the human population in Xiulonkan village in China.

The prevalence of hookworm infection in the present study was found to be high in females than in males in most of the cases. This 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, and therefore, are prone to more infection as compared to the males. Chandler (1926-1928) had made a similar observation in his study of the hookworm infection status in the northeastern region of British India. Gandhi et al. (2001) also

Table VI. Prevalence of hookworm and its associated gastrointestinal nematode infections in Meghalaya post treatment

	3 weeks		5-7 weeks		9 - 12 months	
	No. infected	Prevalence %	No. infected	Prevalence %	No. infected	Prevalence %
Nongkya						
Hookworm	5	1.17	18	4.23	20	4.70
Ascaris	21	4.94	64	15.05	70	16.47
Trichuris	1	0.23	2	0.47	4	0.94
H+A	-	-	16	3.76	20	4.70
H+T	-	-	-	-	-	-
A+T	-	-	-	-	-	-
H+A+T	2	0.47	5	1.17	7	1.64
Total	29	6.82	105	24.70	121	28.47
Sutnga						
Hookworm	29	4.93	41	6.97	48	8.16
Ascaris	25	4.28	74	12.58	116	19.72
Trichuris	-	-	-	-	3	0.51
H+A	3	0.51	11	1.87	17	2.89
H+T	-	-	-	-	1	2.89
A+T	-	-	-	-	1	0.28
H+A+T	-	-	-	-	-	-
Total	57	8.14	126	18	186	26.57
Williamnagar						
Hookworm	44	4.57	81	8.41	85	8.83
Ascaris	41	4.26	79	8.21	103	13.18
Trichuris	-	-	-	-	-	-
H+A	5	0.71	11	1.14	37	4.73
H+T	-	-	-	-	1	0.10
A+T	-	-	1	0.12	2	0.25
H+A+T	-	-	-	-	2	0.25
Total	90	9.35	172	17.87	230	23.90

reported that females were more likely to have moderate or heavy infections, whereas the males were more likely to have light infections. The study also indicated that the infected subjects harboured a combination of geohelminthic nematodes, viz., *Ascaris, Trichuris* and hookworm. Intestinal parasitoses involving multiple species infections are of common occurrence in South-east Asia (Carrol and Walker, 1990; Bakta et al., 1993), the rainforest region of Ramonafana, Madagascar (Kightlinger et al., 1995), in Pemba island in Zanzibar (Renganathan et al., 1995), rural population of Bali (Widjana and Sutisna, 2000), and in the indigenous people in Lushui county of Yunnan province, China (Lili et al., 2000). 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, these two places relate to semi urban epidemiology as they include coal miners and animal farmers (Udonsi, 1983). 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. In an earlier study made elsewhere in the state, Yadav and Tandon (1989) reported a high prevalence of hookworm eggs or larvae in places that are frequently exposed to children. These findings can also be related with the findings of other workers; Toma et al. (1999) identified the age group 4-14 years to be the 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 age (Bundy et al., 1992) and the worms tended to be more aggregated among adults (Bundy et al., 1987; Bundy, 1988; Bradley et al., 1992, Lili et al., 2000; Gandhi et al., 2001).

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 imprecise (Bundy et al., 1992); egg counts may also vary from day to day within individuals (Hall, 1982) and the fecundity of female worms is also dependent on worm density (Anderson, 1986). 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 anemia conditions (Chakma et al., 2002).

After treatment with single dose of mebendazole, the prevalence of infection showed a decline, which indicates that the dose regimen was effective, leading to decrease in the prevalence of infection with hookworm species (Shield et al., 1981). Though the prevalence initially declined, it 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 reinfection or predisposition to infection (Quinnell et al., 2001). It, therefore, emerges from the present study that the control of transmission of hookworm infection cannot be achieved and sustained without intensive health education, together with promotion of environmental health through community involvement in building proper sanitation facilities in the affected rural areas.

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