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BANCROFTIAN FILARIASIS IN TRIBAL POPULATION OF BANKURA DISTRICT, WEST BENGAL, INDIA

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Abstract: The state of West Bengal, India is endemic for bancroftian filariasis. But no information is available on filarial epidemiology among the tribal population of this state. Filariasis survey was conducted during night covering 1,386 tribal people of Banpura district, West Bengal. Indoor-resting mosquitoes were collected from human habitations of the tribal areas and dissected. Microfilaria rate, mean microfilarial density, disease rate and endemicity rate were 3.3%, 6.3%, 5.6% and 8.6% respectively. Per man-hour density, infection and infectivity rates of the vector *Culex quinquefasciatus* were found to be 9.0%, 2.7% and 0% respectively.

Key words: Bancroftian filariasis, microfilaria, tribal population, *Culex quinquefasciatus*

INTRODUCTION

Lymphatic filariasis is a major health problem of particular importance to India. According to WHO (1994), a total of 45.5 million persons are suffering from lymphatic filariasis in Indian subcontinent. Studies on filariasis in West Bengal were mainly restricted to the non-tribal population of Calcutta and its suburbs (Rao and Sukhatme, 1941; Rozeboom *et al.*, 1968; Bhattacharya and Gubler, 1973; Hati *et al.*, 1989; Chandra *et al.*, 1994) except few in other areas (Iyenger, 1941; De and Chandra, 1994; Chandra and Hati, 1996). No work was conducted so far having information about the filarial situation in tribal population of this state. The present study has been formulated to gather first hand information on filariasis among the tribals and the vector status in tribal populated areas of Bankura district, West Bengal, India.

MATERIALS AND METHODS

A survey was conducted covering 15% of the total population of 8 tribal villages namely Sukhosol, Dakhinasol, Baishnabbandh, Burroangari, Deldanga, Siberbandh, Nimdanga and Nabinnagar within a radius of 10 km around Sonamukhi Municipal town of Bankura district of West Bengal, India. A total of 1,386 tribal individuals of Santals, Kora and Munda classes were examined during the survey. These tribal classes are included in the racial group of kolid usually found in the North Deccan forests. They have strong totemistic beliefs. Matriarchal influence is observed. Skin colour is black brown (Das, 1988). Prior permission was taken by holding discussions with respective village heads and family members about the purpose of the study before survey. The survey was carried out between 19:00 hr to 22:00 hr. A blood sample of 20 μl was collected from all the family members by finger prick (Gubler *et al.*, 1973) and thick blood smear was prepared. Then the slides were examined for the detection of microfilariae (Mf). Signs and symptoms of bancroftian filariasis from both sexes were recorded through physical examination. Age, sex and clinical history of each subject were also noted. Symptoms like filarial adenolymphangitis and epididymo-orchitis are accompanied by filarial fever and both of them often recur several times a year. Incidence of recurrence was recorded during taking the clinical history. Permanent oedema, which does not subside, without any apparent change of the skin is classified as lymphoedema. When the skin becomes chronically thickened along with hypertrophy of the sub-cutaneous tissues it is classified as elephantiasis.

For mosquito collection 16 spots were selected in 8 tribal villages (2 spots in each village). 10 human

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habitations were chosen from each spot and indoor resting mosquitoes were captured between 06:00 hr to 08:00 hr for 12 min from each habitation once a week from August 1994 to July 1995. Thus a total of 8 man-hours were employed in each month for mosquito collection following the method of Holstein (1954) and WHO (1962). In the first week mosquitoes were collected from the habitations of spot no. 1, in the second week from the spot no. 2 and so on. Thus collections were made serially from spot no. 1 to 16 in one season (one season includes 4 months i. e. 16 wks). Collections were made in all the seasons of the year namely rainy (Jul-Oct), winter (Nov-Feb) and summer (Mar-Jun) following the method of De and Chandra (1994). A total of 160 fixed human habitations were searched for mosquitoes in each season. Mosquitoes were dissected and examined to determine the prevalence *W. bancrofti* larvae. Statistical analysis were computed with the help of Normal deviate 'Z' (to compare rates) and Student's 't' test (to compare averages).

Results and Discussion

Parasitological and clinical studies

The survey revealed an overall Mf rate, Mf density (Average load of Mf per positive person per 20 μl blood), disease rate and endemicity rate (rate of Mf carriers plus clinical cases; Mf positive clinical cases have been considered once) of 3.3%, 6.3%, 5.6% and 8.6% respectively in the present study area. Out of 8 villages surveyed only 4 were found to be with Mf positive patients, but diseased persons were recorded in all the villages. Variation in endemicity rate of filariasis was noted according to villages and endemicity rate was higher in those villages which were close to the urban areas.

Mf rate among the tribal population of the present study area (3.3%) was almost same to that of the tribes of Panna district (3.8%), Madhya Pradesh (Chand *et al.*, 1996) and much lower than that of the tribes of Nancowry group of islands (11.9%) in Andaman and Nicobar islands (Tewari *et al.*, 1995). Information on filarial endericity among the tribes of India is very limited.

Age and sexwise details are shown in Table 1. Both Mf rate and Mf density among males (4.4%; 7.7) were higher ($p<0.05$) than those in females (2.2%; 3.6) of the present study area as also found in Panna district. Though overall Mf rate was not very high in tribal villages of Bankura district, it was higher in the working age groups specially between 31 and 40 years in both male (8.4%) and female (5.8%). The time of blood collection between 19:00 and 22:00 hr was slightly early for nocturnally periodic strains which might cause slight underestimation in Mf rate. This is worthy mentioning that there are no electricity facility in the isolated tribal villages and so the tribal people go to bed within 8 p.m. usually. Male children below 10 years did not show Mf positivity. Mf density was noted to be the highest in the age group of 11-20 years both in males (10.1%) and females (7.0%) indicating that the people of tribal community of the study area became infected with filarial worms in their earlier age. Moreover, the existence of two female Mf positives at age≦10 is a direct finding to support the idea of early age infection among tribals.

Different clinical signs and their age and sexwise distribution are presented in Table 2. Overall disease rate (5.6%) was higher ($p<0.05$) than the Mf rate (3.3%). Out of 78 clinical patients 5 (6.4%) were Mf positive. Disease rate among males (8.0%) was also higher ($p<0.05$) than that in females (3.2%). Disease

Table 1 Microfilaria rate, Mf density and disease rate according to sex and age group among tribal villagers

Age group (Years)	Male				Female				Total			
	No. examined	Mf rate (%)	Mf density	Disease rate (%)	No. examined	Mf rate (%)	Mf density	Disease rate (%)	No. examined	Mf rate (%)	Mf density	Disease rate (%)
≦10	142	0	0	0	134	1.5	6.5	0	276	0.7	3.2	0
11-20	215	6.5	10.1	8.4	194	1.0	7.0	1.0	409	3.9	8.5	4.9
21-30	154	3.9	6.3	9.1	133	1.5	4.5	7.5	287	2.8	5.4	8.4
31-40	95	8.4	5.2	15.8	104	5.8	1.5	3.8	199	7.0	3.3	9.5
41-50	55	3.6	6.0	14.5	68	2.9	3.5	5.9	123	3.3	4.7	9.8
51-60	31	3.2	5.0	3.2	46	2.2	3.0	4.3	77	2.6	4.0	3.9
61-70	10	0	0	0	5	0	0	0	15	0	0	0
Total/ Avg	702	4.4*	7.7*	8.0*	684	2.2*	3.6*	3.2*	1,386	3.3*	6.3*	5.6*

Table 2 Different filarial symptoms i. e adenolymphangitis, lymphoedema, elephantiasis, epididymo-orchitis and hydrocele according to sex and age group among tribal villagers

Age group (Years)	Adenolymphangitis				Lymphoedema				Elephantiasis				Epididymo-orchitis		Hydocele		Total clinical symptom(s) of any sort					
	Male		Female		Male		Female		Male		Female		Male		Male		Male		Female		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
≦10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11-20	0	0	0	0	2	0.9	2	1	0	0	0	0	7	3.3	9	4.2	18	8.4	2	1	20	4.9
21-30	7	4.5	7	5.3	0	0	3	2.3	0	0	0	0	3	1.9	4	2.6	14	9.1	10	7.5	24	8.4
31-40	3	3.2	4	3.8	0	0	0	0	0	0	0	0	3	3.2	9	9.5	15	15.8	4	3.8	19	9.5
41-50	2	3.6	3	4.4	0	0	0	0	0	0	1	1.5	0	0	6	10.9	8	14.5	4	5.9	12	9.8
51-60	0	0	2	4.3	0	0	0	0	0	0	0	0	0	0	1	3.2	1	3.2	2	4.3	3	3.9
61-70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total/Avg*	12	1.7*	16	2.3*	2	0.3*	5	0.7*	0	0	1	0.1*	13	1.9*	29	4.1*	56	8.0	22	3.2	78	5.6

rate was higher in the working age groups (21-50 years) both in males and females. Elephantiasis in the leg was noticed in a 47 year old woman only.

Mosquito study

A total of 2,216 female mosquitoes of 7 species were collected which included *Culex quinquefasciatus* (39.1%), *Cx. vishnui* group (13.9%), *Anopheles subpictus* (10.0%), *An. vagus* (27.6%), *An. barbirostris* (7.6%), *Armigeres subalbatus* (1.2%) and *Mansonia indiana* (0.6%). *Cx. quinquefasciatus* was incriminated as the vector of *W. bancrofti* in these tribal villages. Average per man-hour density of *Cx. quinquefasciatus* was 9.0 (ranging from 7.0 to 11.3 in different months). Availability of vector species in human habitations showed no seasonal ($p > 0.05$) variations as indicated by the prevalence in rainy season (37.8%), winter (34.9%) and summer (27.2%). Out of total 23 infected *Cx. quinquefasciatus*, 13 (56.5%), 6 (26.1%) and 4 (17.4%) were found to carry Mf, 1st stage and 2nd stage larvae of *W. bancrofti* respectively. No 3rd stage larva was detected in the wild vector population. Overall vector infection rate was 2.7% (ranging from 0% to 4.7% in different months). Seasonal vector infection rates were 3.0%, 2.3%, and 2.5% in rainy season, winter and summer respectively without ($p > 0.05$) any marked variation. Vector infection rate and infectivity rate (2.7%, 0%) in the present study area were lower than those of Panna district (4.9%; 1.0%).

Life style of tribals and vector mosquito

Less intensity of filarial infection among females is common in all the endemic places in India as they wear more cloths and are less exposed to mosquito bites. As the tribes are labour class poor people, all the family members except old age people work in the field. They are usually not provided with employment in their own villages. So they used to spend a long time of the year in non-tribal areas for employment with family and children. Low vector infectivity rate (0%) indicates that transmission cycle of filarial worm is not very active in the tribal villages. Probably they carry the infection from some highly endemic places outside where they used to go for cultivation work, indicating a correlation of higher infection among working age groups. Tribal people generally live in moderate or dense forests usually isolated from urban areas. They maintain a separate socio-cultural heritage and are more close to nature. In stead of ill developed sanitary structures, associated with indiscriminate urbanization (commonly found in the non-tribal areas), the tribes keep their houses and surroundings in such a manner (neat and clean) as to be protective against vector breeding. Man-hour density of vector species in the tribal areas is much lower than those of the non-tribal areas studied so far in West Bengal. Informations gathered during the presest work will be of immense help to formulate an effective control strategy. Use of bed nets during the 3rd quadrant of night (12 midnight to 3 a. m.) as suggested by Chandra (1995) will be useful for protection against infection specially when they stay in non-tribal areas for work. Health education through community participation may be useful along with chemotherapy and vector control.

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GIARDIA-INFECTION AMONG SUBJECTS REVEALED DIARRHEA OR PASSAGE OF SOFT STOOL IN KOCHI PREFECTURE, JAPAN

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Abstract: Prevalence of *Giardia* spp. infection was determined among subjects attending Kochi Medical School Hospital from April 1996 to January 1998. Cysts of *Giardia* spp. were found in 10 (0.6%) out of 1,702 stool samples examined. Infection rate was higher among aged males. Most of the subjects with *Giardia* organisms had no history of traveling overseas except 2 cases. Although source of infection was not determined, most of the positive subjects supposed to be infected within the community.

Key word: *Giardia*, Giardiasis, Diarrhea, Soft feces, Advanced age group

INTRODUCTION

Giardiasis is one of the common intestinal protozoan infections. This disease is prevailing in developing countries (Tompson *et al.*, 1993). Even in developed countries, infections with *Giardia* spp. have been reported from day-care centers (Pickering *et al.*, 1981). Food- borne transmission (Peterson *et al.*, 1988) and water-borne transmission (Moorehead *et al.*, 1990) have also been reported. Reflecting recent advancement in international interchange and traveling abroad, imported cases have been increasing in Japan. In addition, a close association between sexually-transmitted diseases and protozoan infection has been focused (Takeuchi and Miyahira, 1989). To date, several data on prevalence of giardiasis diagnosed by stool examination have been reported by health screening and from admitted patients in different 2 hospitals in metropolitan area and Tokai region in Japan (Kado *et al.*, 1986; Ichizawa *et al.*, 1990). However, a few cases regarding the disease have been reported from Shikoku region, especially from Kochi Prefecture, which is different from other metropolitan area in socio-economics as well as population structure including life styles of the people.

SUBJECTS AND METHODS

Study subjects

Stool samples were collected from 1,702 subjects of 0-94 years of age who visited the Kochi Medical School Hospital either as admitted or outpatients from April 1996 to January 1998. These subjects included 950 males and 752 females who had either history of diarrhea or passage of soft stool.

Stool examination

Fecal specimens were examined microscopically by the direct smear method for trophozoites or cysts of *Giardia* spp. To confirm *Giardia*-positive cases, they were processed by Wright's solution or modified Kohn' s one-step staining (Gleason and Healy, 1965). Smears from diarrhea samples were also stained with modified Ziehl-Neelsen carbol-fuchsin to detect *Cryptosporidium* oocysts (Henriksen and Pohlenz, 1981).

Examination of blood

Blood was tested for peripheral blood cell counts, immunoglobulin assay and biochemical analysis before and after the treatment in patients with *Giardia* parasites. Particle agglutination tests (Fujirebio, Tokyo, Japan) were performed for the measurement of serum antibody against human T-cell lymphotropic virus-type

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Table 1 Age and sex-distribution of subjects examined for *Giardia* infection

		Male		Female	
Age (years)	Total examined	Negative	Positive (%)	Negative	Positive (%)
≦10	87	55	0 (0.0)	32	0 (0.0)
11-20	70	31	0 (0.0)	39	0 (0.0)
21-30	73	34	0 (0.0)	39	0 (0.0)
31-40	79	37	0 (0.0)	42	0 (0.0)
41-50	188	90	0 (0.0)	98	0 (0.0)
51-60	257	133	2 (1.5)	121	1 (0.8)
61-70	507	308	5 (1.6)	194	0 (0.0)
71-80	361	214	1 (0.5)	146	0 (0.0)
≧81	80	39	1 (2.6)	40	0 (0.0)
Total	1,702	941	9 (1.0)	751	1 (0.1)

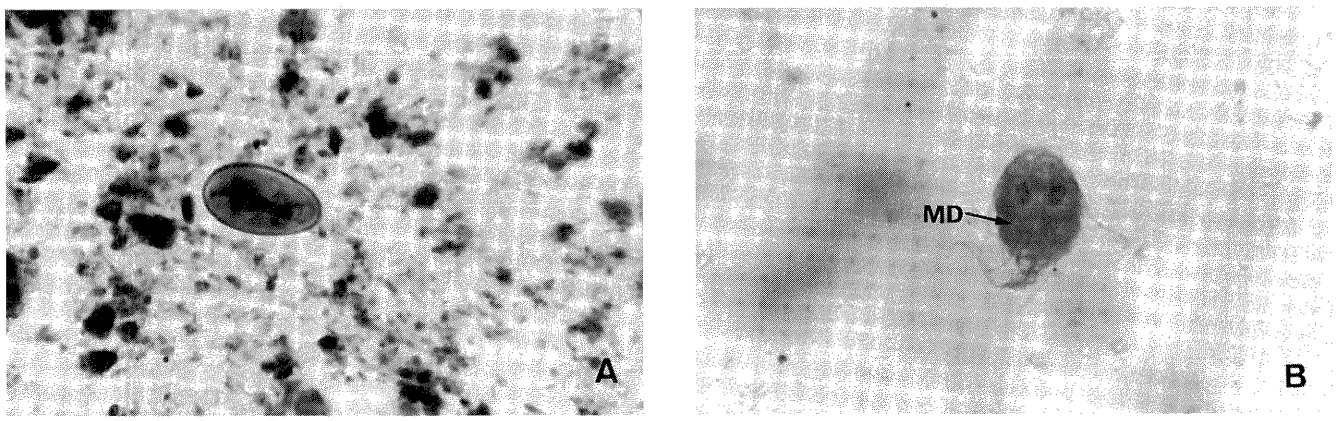
I (HTLV-I).

Statistical analysis

Contingency table analysis was used to determine the probability of significant differences between the sex and age groups. Paired t test was applied to evaluate hematological data. A value (P) of less than 0.05 was considered significant.

Results and Discussion

The prevalence rate of positive cases for *Giardia* organisms is shown by sex and age in Table 1. Out of 1,702 subjects examined in the present study, stool samples from 10 subjects (0.6%) consisting of 9 males and 1 female were positive for cysts of *Giardia* by microscopic examination. In most of the cases, only cysts of *Giardia* spp. were found in fecal samples. Trophozoits were observed in fecal samples from 2 cases and bile sample from one patient. The species of the genus *Giardia* have been reported to be divided into three groups (*G. agilis* group, *G. muris* group and *G. duodenalis* (=*G. intestinalis*=*G. lamblia*) group) differing in the shape and position of the median bodies, the shape of the cell, and the relative length of the adhesive disk (Kulda and Nohýnková, 1978; Tompson *et al.*, 1993). The morphology of the trophozoites observed was pyriform in shape (body length; 13.6±1.3 μm, adhesive disk length; 6.3±0.6 μm, n=30) and the median body was situated approximately across the body (Fig 1). Based on these morphological features, the organisms were identified as trophozoites of *Giardia lamblia*. To date, it is not possible to distinguish morphologically the cyst of *G. lamblia* from that of other *Giardia* spp. Therefore, it should be necessary to develop a molecuar genetic analysis, using PCR, in the future to identify the species of the cysts in the fecal samples. In this study, *Cryptosporidium* and other protozoa were not found in diarrheal stool samples examined. The characteristic



(A) (B)

Figure 1 (A) Cyst of *Giardia* stained with modified Kohn's one-step staining (×1,000). (B) Trophozoite of *G. lamblia* Stained with Wright's solution. The median body (MD) is situated approximately across the body (×1,000).

Table 2 Summary of the subjects with *Giardia* organisms

No.	Age (years)	Sex	Travel History*	Detection of trophozoites	Symptoms†	Clinical History‡	Drug§
1.	69	M	SEA	−	Abdominal pain, ailment	Cancer (tongue)	N
2.	72	M	NA	−	NA	Cardiac disease, Diabetes mellitus	NA
3.	61	M	N	−	Diarrhea, Abdominal ailment	Cancer (lung)	CD
4.	68	M	N	+	N	Cancer (lung)	N
5.	60	M	N	+	Diarrhea, Abdominal ailment	Cerebral infarction	N
6.	67	M	NA	−	NA	Cancer (mesopharynx), Pneumonia	CD
7.	60	F	N	−	N	Plasmacytoma	CD
8.	81	M	Eur	−	N	Diabetes mellitus, Gout	N
9.	70	M	N	−	N	Cardiac disease	N
10.	58	M	NA	−	N	Cardiac disease	N

*Eur=Europe; N=none; NA=no answer; SEA=Southeast Asia.
† Main symptoms relating to giardiasis
‡ Main history
§ Administration of immunosuppressive drug or carcinostatic drugs (CD)

feature of the present study is that *Giardia*-positive cases were distributed in advanced age group (>51 years old). This tendency is not consistent with age distribution in developing countries (Gilman *et al.*, 1985). The prevalence rate was comparable to those reported from Fukuoka, Tokyo and Shizuoka in Japan by Mako (1982), Kado *et al.* (1986) and Ichizawa *et al.* (1990) respectively. Occupation of the subjects with *Giardia* parasites was different.

A few subjects had symptoms related to *Giardia* infection (Table 2). Most of the subjects had history of malignant neoplasm and cardiac diseases, and some had been taking carcinostatic drugs. None of the subjects was infected with HTLV-I, although the disease is endemic in Kochi (Taguchi *et al.*, 1983). One out of 8 subjects with *Giardia* parasites was resistant to the treatment with metronidazole. It has been reported that drug resistance in giardiasis is related with agammaglobulinemia, intestinal hyposecretion of IgA, hypoacidity and etc. (Wolf 1975; Akahori *et al.*, 1994). In the present metronidazole-resistant case, however, a serum immunoglobulin level was normal. It has been suggested that host's cellular cytotoxicity for the parasite is involved in chronic giardiasis (Smith *et al.*, 1982). Monocyte counts in peripheral blood of several subjects positive for *Giardia* decreased after treatment with metronidazole (Fig 2). Monocyte-derived macrophages have been reported to have capacity to ingest *Giardia* trophozoites and to kill intracellular parasites (Hill and Pearson, 1987), although it remains to be solved that monocytes are important effector cell *in vivo*.

Fecal examination was done of all the member of a family living with one parasitologically-confirmed subject. None of the family members had been found

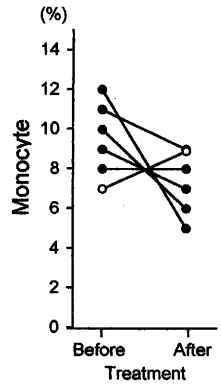


Figure 2 Monocyte rates in peripheral blood from subjects with *Giardia* organisms before and after treatment with metronidazole. Statistical analysis excluded data of which one patient (open circle) persisted in passing cysts of *Giardia* in his stool after treatment.

infected with *Giardia*. Most of the parasite-positive subjects had no pet animal. At least some isolates of *Giardia* have a wide range of host susceptibility. Therefore, it is possible that humans and a variety of animals naturally share the parasite (Baker and Gerschwin, 1987; Collins *et al.*, 1987; Thompson 1992). Two subjects positive for the organism went abroad within last several years. Most of the positive cases in the present study were thought to be infected within their community, but the source of infection was not determined.

In conclusion, we showed the higher prevalence of *Giardia* spp. infection in advanced age group, when stool samples from subjects of 0 to 94 years of age were examined. Such a tendency might be caused by an immunological condition of the host subjects such as immunodepression by aging. Nowadays, in Japan, the

ratio of advanced age group ha been increasing especially in Kochi Prefecture. Therefore, more attention should be given to such parasitic infections at laboratory stool examinations, in order to eliminate cyst carriers as potential source of the infection.

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Research Note

ANTIFILARIAL EFFECT OF A PLANT *CARICA PAPAYA*

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Abstract: Air-dried and powdered seeds of unripe *Carica papaya* was administered orally at 60 mg/kg/day for 30 days on four pariah dogs naturally infected with *Dirofilaria immitis*. The treatment resulted in 80% reduction in microfilarial density on day 30 following the onset of treatment. Microfilarial density rose gradually and the level of reduction in the sampling on day 180 was 52%. *In vitro*, the drug did not produce any lethal effect on the microfilariae of *D. immitis*. The prolonged maintenance of the reduced level of microfilarial density may be due to the sizeable reduction of adult worm loads.
Key words: *Carica papaya*, antifilarial, *Dirofilaria immitis*

The drug of choice for treating filarial infection, diethylcarbamazine is a good microfilaricide, but produces serious side reactions. With respect to its efficacy as macrofilaricide, the drug may be required to be given at 6-8 mg/kg/day for a prolonged period i.e. 1 week each month for 6 to 12 months (Ottesen, 1985). Ivermectin given as a single dose (200 to 400 μg per kg) would affect a decrease in microfilaraemia comparable to that of DEC, but the efficacy of ivermectin as macrofilaricide has not yet been determined (Ottesen, 1993). The WHO special programme has given the highest priority to the development of new antifilarial drugs particularly macrofilaricide or drugs against the adult filarial parasites (TDR, 1991). The seeds, roots and leaves of *Carica papaya* L. (Caricaceae) have long been used as anthelmintic, antiseptic, diuretic from time immemorial (Blatter *et al.*, 1933). The plant, a small soft-wooded, fast growing and short-lived tree is cultivated throughout the country. The plant is known to contain heat and acid-tolerant low molecular weight proteins, benzyl isothiocyanate, potassium benzyl glucosinolate and the proteolytic enzyme papain. The antioxidative property of unripe papaya has been attributed to its vitamin C content, malic acid, citric acid and glucose (Osato *et al.*, 1993). The anthelmintic activity of papaya against *Heligmosomoides polygyrus* was studied in experimentally infected mice (Satrija *et al.*, 1995). Four groups of mice were given latex at dose levels of 2,4,6 and 8 g/kg body weight which showed an antiparasitic efficacy of 55.5, 60.3, 67.9 and 84.5%, respectively. With a view to explaining scientifically the anthelmintic property of papaya, we studied its antifilarial activity on dogs naturally infected with *Dirofilaria immitis*.

The seeds of unripe papaya were dried in the shade and ground. The powdered materials were used for *in vitro* and *in vivo* tests. Initially we have used the whole plant material and not its solvent extracts because that latter may leave some effective components.

A sample of blood was collected by means of a sterile syringe from a microfilaraemic dog. The sample was kept at 37°C for 6 h and the serum (1 m*l*) containing 2,530±311 (Mean±SE) microfilariae was transferred to a cavity block, then mixed with 2 mg of test material and kept under stirring for 10 min. The vitality of *D. immitis* microfilariae was evaluated after 120 min of incubation at 37°C (Chakraborty *et al.*, 1996). The serum was used in the *in vitro* only to avoid the osmotic stress which the larvae might encounter in other media. In our previous study we kept the duration of exposure as 2 h (Dutta and Sukul, 1982).

Blood was sampled from 8 microfilaraemic dogs naturally infected with *D. immitis* (3 males and 5 females) every week for a period of 10 weeks and microfilarial density per 20 mm^3 blood was determined for each sample. The blood film was allowed to dry,

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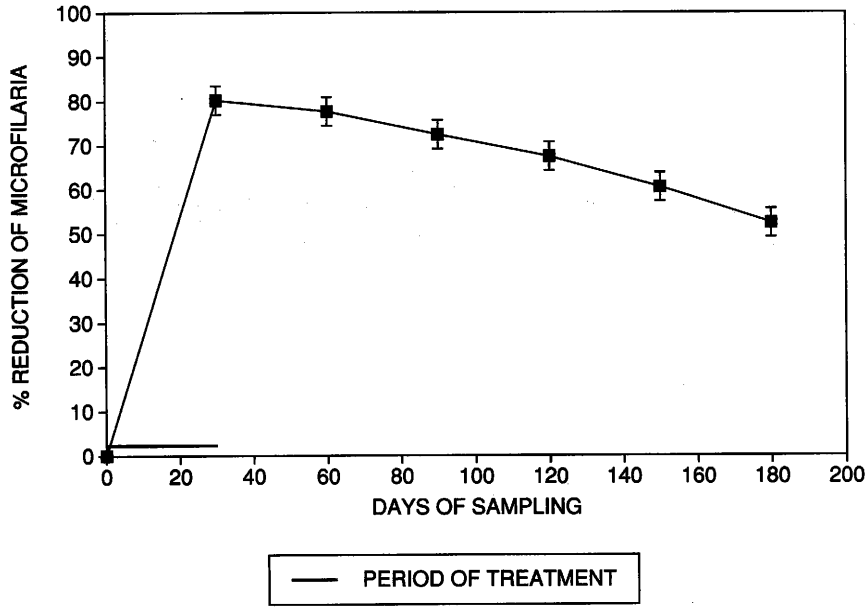

Figure 1 Percentage reduction in microfilarial concentration in dogs treated with the powdered seeds of *Carica papaya*.

dehaemoglobinised in distilled water and stained with Giemsa stain. After determining the microfilarial concentration for 10 weeks, 4 microfilaraemic dogs (2 males and 2 females) were orally administered the powdered material of *C. papaya* at 60 mg/kg/day. The treatment was given once daily for 30 days. The remaining four microfilaraemic dogs (1 male and 3 females) served as controls. Empty capsules were filled with the drug, placed inside a loaf of bread and then offered to the infected dogs (Sarkar *et al.*, 1997). Control dogs received empty capsules in a loaf of bread. Dogs were continuously kept under observation until they swallowed the loaf containing capsules and took more pieces of bread offered thereafter. Blood was first sampled on day 30 from the date of treatment. Additional samplings were done at monthly intervals up to 180 days.

No change in the vitality of *D. immitis* microfilariae occurred after an exposure of 120 min to *C. papaya* seeds at 2 mg/m*l*.

The mf counts per 20 mm^3 of blood in the 8 dogs before treatment are shown in Table 1. The mf concentration in the 8 dogs did not vary appreciably during the 10-week period of observation before treatment. The percent reduction in mf count following treatment with the *C. papaya* seeds is plotted against days of sampling and of treatment in Figure 1. The mf density showed a 80% fall on day 30 following the onset of treatment as compared to the pre-treatment level (Fig. 1). Thirty days after the last date of treatment, a 77% fall was observed with respect to the same initial density (Fig. 1). Thereafter, the mf count started rising very slowly, and even 150 days after the treatment 52% reduction

Table 1 Microfilarial concentration per 20 mm^3 blood at weekly intervals

Dogs	Weekly counts of microfilariae												
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Mean	S.D.	S.E.
Female	4,590	4,423	4,670	4,702	4,431	4,399	4,555	4,477	4,410	4,533	4,519	109.4	±34.6
Female	3,729	3,864	3,755	3,816	3,521	3,902	3,929	3,880	3,630	3,580	3,761	143.1	±45.2
Female	2,970	2,537	2,789	2,598	2,864	3,052	3,093	2,664	2,923	2,936	2,942	286.2	±95.4
Female	2,225	2,283	1,972	2,175	2,469	2,602	2,322	2,463	2,137	2,069	2,271	196.6	±65.5
Female	3,933	3,877	3,989	3,623	3,661	3,728	4,244	4,301	4,175	4,059	3,959	239.9	±79.9
Male	4,110	4,280	4,315	4,301	4,017	4,426	3,897	3,876	3,733	4,015	4,097	227.4	±75.8
Male	3,212	3,372	3,379	3,312	3,258	3,111	3,115	3,391	3,179	3,192	3,251	108.2	±34.2
Male	3,668	3,602	3,747	3,769	3,502	3,533	3,574	3,688	3,671	3,542	3,634	86.8	±27.4

was maintained. Since the plant material is edible and effective through the oral route it is relatively a more promising antifilarial agent as compared to other plants reported earlier by Datta and Sukul (1987) and Chakraborty *et al.* (1996). The treated dogs did not show any apparent toxicity in terms of change in body weight, temperature, intake of food and movement.

The marked reduction in the microfilarial density following 30 days of treatment indicates that the seed of unripe papaya is a very effective microfilaricide. The post treatment maintenance of the reduced level of microfilarial concentration in blood suggest that some of the adult worms might have been killed by the drug. The treated dogs could not be sacrificed for adult worm. The mixture of leaf, seed and pulp of unripe papaya has been shown to produce antimicrobial and antioxidant property (Osato *et al.*, 1993). However, the antioxidant property was more than 10-fold higher than its microbial activity. The benzyl isothiocyanate obtained from papaya is suggested to possess antimicrobial activity. Its antioxidant property is attributable to its vitamin C content, malic acid, citric acid, glucose and monosaccharides. This precludes the possibility of explaining the mechanism of action of the drug in inducing membrane damage of the parasite through lipid peroxidation. The combined results of *in vitro* and *in vivo* suggest that antifilarial activity of the seeds of unripe papaya may result from the biotransformation of its constituents by the digestive enzymes of the host. Since this drug is non-toxic and effective by oral administration it can be tried against human filariasis in future. The results of the present study suggest that papaya may be effective and important inexpensive clinical agent in eliminating filarial parasite from the hosts. However, further studies are required to determine its value as a potential antifilarial agent.

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