Improvement in the efficacy of existing combination of antifilarials by inclusion of tetracycline in rodent model of brugian filariasis

Preeti Bajpai[1], Kumkum Srivastava[1], Shilpy Shakya[1], Prabhu N. Saxena[2] and Shailja Misra-Bhattacharya[1,*]

[1]Division of Parasitology, Central Drug Research Institute, Post Box No. 173, Lucknow 226 001, India
[2]Department of Zoology, School of Life Sciences, B.R. Ambedkar University, Dr Khandar's Campus, Agra 282 002, India

Newer control strategies like combination drug therapy have proved effective against a variety of pathogens, including filarial parasites. The recent discovery of *Wolbachia*, a bacterial endosymbiont present in the filarial parasites, dependence of parasites on the bacteria for their own survival, and fecundity and bacterial sensitivity to tetracycline have given new hope to the ongoing antifilarial drug development programme, especially in view of the conflicting combination field trial results obtained from various geographical regions. The present studies were therefore planned to investigate whether adding tetracycline to the already recommended combinations of antifilarials would lead to further improved antifilarial efficacy in terms of killing of adult worms or their sterilization. The present study therefore employs the *Brugia malayi/Mastomys coucha* model in order to extrapolate the findings in human filarial patients. Different combinations were used and all of these proved to exert greater micro and macrofilaricidal action. The best combination (diethylcarbamazine + ivermectin + albendazole) exerted remarkable macrofilaricidal efficacy (63%), sterilized all the surviving female parasites and completely eliminated microfilariae from the peripheral blood on day 90 post-treatment. Tetracycline enhanced the embryostatic action of all the filaricides, although it alone did not demonstrate any significant embryostatic efficacy when administered for five consecutive days. The present findings thus strongly suggest the limited clinical trial of tetracycline in combination with the standard filaricides.

Keywords: Albendazole, antifilarials, *Brugia malayi*/mastomys, diethylcarbamazine, ivermectin, tetracycline.

LYMPHATIC filariasis continues to be a worsening problem in the tropics, especially in Africa and the Indian subcontinent[1]. It causes not only high morbidity but also social stigma and economic loss, which are grossly underestimated[2]. Over the last ten years dramatic research advances have led to new understanding about the severity and impact of the disease, new diagnostic and monitoring tools and, most importantly, new treatment tools and control strategies. The single annual or biannual dose therapy with diethylcarbamazine (DEC) or ivermectin[3], the superiority of combination therapy over single drugs[4–7] and the recent inclusion of albendazole in the combination[8] appear to be more effective in controlling human filarial infections. However, issues like possible development of drug resistance against ivermectin and reduced compliance in the face of perceived cure may ultimately result in a return to pre-programme disease prevalence that, without the development of new interventions may progress to present new problems for public health. The recent discovery of *Wolbachia* as a bacterial endosymbiont associated with filarial parasites, has given a new dimension to the drug development programme. The activity of tetracycline, an anti-rickettsial drug, has been reported against a number of filarial species[9,10], indicating the possibility of developing tetracycline as an antifilarial agent[11]. Nevertheless, further trials on different treatment strategies and other antibiotics may exploit this approach in future, and preclude the prohibitively expensive development of new chemotherapeutic compounds. The combination of antibiotics with standard filaricides also needs to be evaluated to identify possible synergistic effects.

Limited human clinical trials, using various combinations of available filaricides, have yielded encouraging results, especially in terms of microfilaricidal efficacy, as there are no markers to detect macrofilaricidal activity of antifilarial drugs when they are used against brugian filariasis. However, it is now possible to determine the adulticidal efficacy against bancroftian infections, using ultrasound or antigen levels[8,12]. Therefore, macrofilaricidal effects of any drug used against brugian infections are based on indirect evidences. Parallel animal studies are therefore necessary to evaluate the actual results of a single or a combination of drugs on adult brugian parasites. The present study was therefore planned to explore the microfilaricidal, macrofilaricidal and female worm sterilization efficacy of different combinations of DEC, ivermectin, albendazole and tetracycline over each drug alone.

Male *Mastomys coucha*, 6–8-week-old (GRA 'Giessen strain') subcutaneously infected with 100 infective larvae (L_3) of *Brugia malayi* were used as experimental hosts[13]. The animals were divided into two groups, one consisting of 74 animals, which received higher dose levels and the other comprising 50 animals to which lower doses were administered. Each treated group contained six animals and the untreated control group had eight animals each. The treatment strategy included various drug combinations, viz. DEC + ivermectin (DI); DEC + albendazole (DA); ivermectin + albendazole (IA); DEC + ivermectin + albendazole (DIA); DEC + tetracycline (DT); albendazole + tetracycline (AT); ivermectin + tetracycline (IT); DEC + ivermectin + tetracycline (DIT); DEC + albendazole + tetracycline (DAT); ivermectin + albendazole + tetracycline (IAT); DEC alone (D); albendazole alone (A); ivermectin

*For correspondence. (e-mail: shailja_cdri@rediffmail.com)

alone (I); tetracycline alone (T) and untreated control (UN). DEC was used at 50 mg/kg, albendazole at 200 mg/kg, tetracycline at 200 mg/kg and ivermectin at 2 mg/kg. All the drugs except ivermectin were administered orally for five consecutive days. Ivermectin was injected subcutaneously in a single shot.

The efficacy of various drug formulations on filarial parasite was determined on the basis of microfilaraemic load in systemic circulation of the drug-treated animals, as reported earlier[14]. Briefly, an aliquot of 10 µl blood sample was taken from tail vein of each animal on day 0, 15, 30 and thereafter every fortnight till day 90 post-treatment. Microfilaricidal (MIF) efficacy was determined by per cent change in microfilaraemic density compared to the pre-treatment count. The animals were sacrificed after day 90 post-treatment and various organs, viz. heart, lungs, lymph glands and testes were taken out and gently teased to isolate adult parasites. The parasites were examined microscopically for their number, sex and general condition. All the female parasites were dissected and further examined microscopically to observe the effect of drug on the female reproductive system. Student's *t*-test was used to analyse the statistical significance of the data.

All the drug combinations demonstrated superior MIF efficacy than any of the drugs alone. In bancroftian filariasis, DEC in association with ivermectin has been reported to bring about 90% reduction in microfilarial levels one year post-treatment and low recurrence on annual single-dose treatment with no mention of any adulticidal action[4]. Addition of ivermectin to diethylcarbamazine provided greater reduction in microfilarial prevalence and density than the addition of albendazole[15]. However, ivermectin is available only in African countries and therefore, it is imperative to include albendazole in all other areas where lymphatic filariasis elimination programmes are being implemented. Our findings also indicate an almost parallel MIF efficacy (Table 1). The MIF efficacy of this combination seems to be mostly DEC-mediated, since DEC alone brings about 45.5% of worm death (Figure 1). The embryo-static effect observed appears to be totally exerted by ivermectin which alone revealed more than a 95% sterilizing efficacy, thus demonstrating no synergistic efficacy of the combination (Figure 2).

The DEC–albendazole combination shows efficacy close to the DEC–ivermectin combination in terms of microfilarial (Table 1) as well as adult worm killing (Figure 1), the combination preferred in India over DEC–ivermectin because of its cost-effectiveness, easy availability of albendazole and its additional effect on intestinal parasites[16]. Albendazole, unlike DEC is not a microfilaricidal drug. However, it greatly decreases microfilarial density over several months, presumably because of its macrofilaricidal activity[17]. Absence of reaction after multidrug administration with albendazole will significantly improve acceptability of the control programme. Helminths are potent immuno-modulators and mediate T-helper-2-polarized T-cell responses and potentially increase susceptibility of infected hosts to several intracellular parasitic diseases such as malaria, tuberculosis and AIDS[18]. Annual administration of albendazole leading to deworming the population could thus have far-reaching benefits other than filariasis control[19]. Administration of albendazole is safe, whereas diethylcarbamazine citrate alone or with albendazole results in a significant proportion of patients reporting reactions[8,20]. The public-health campaign for multidrug administration with albendazole thus needs to be made more broad-based to improve acceptability of the programme among the population and health administrators[21].

Albendazole also led to marginal improvement in the MIF efficacy of ivermectin (Table 1). However, significant ($P < 0.05$) decrease in the embryo-static efficacy of ivermectin was observed (Figure 2). However, a recent report on the clinical trial of a combination of ivermectin and albendazole[22] revealed 100% MIF efficacy. Albendazole in repeated high doses has been shown to be macrofilaricidal in bancroftian filariasis[23]. However, it did not demonstrate any apparent effect on adult *B. malayi* in our study when used alone at 200 mg/kg. Rather, it seemed to

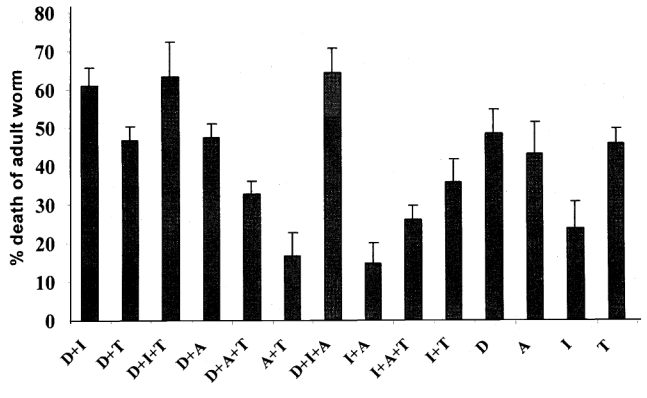

Figure 1. Macrofilaricidal efficacy of various combinations of diethylcarbamazine (D), albendazole (A), ivermectin (I) and tetracycline (T) against *Brugia malayi* in *Mastomys coucha.*

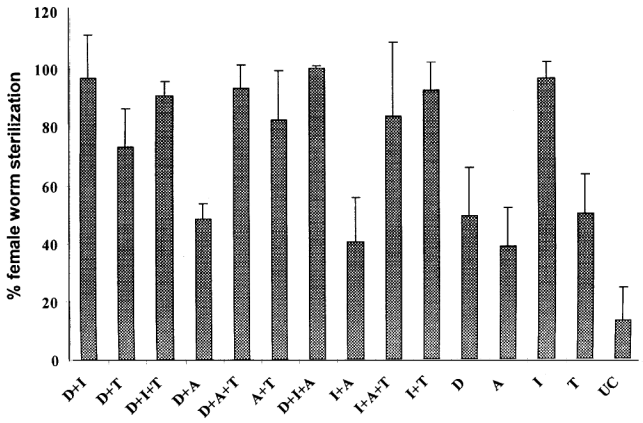

Figure 2. Embryo-static efficacy of the combination of diethylcarbamazine (D), albendazole (A), ivermectin (I) and tetracycline (T) against *B. malayi* in *M. coucha.*

Table 1. Microfilaricidal efficacy of various combinations of antifilarials and tetracycline against *Brugia malayi* in *Mastomys coucha*

Drug combination	Dose schedule	Per cent microfilariae decline in 10 µl of tail blood							
		8 d	15 d	30 d	45 d	60 d	75 d	90 d	105 d
DEC + ivermectin	50 mg/kg p.o.x5d + 2 mg/kg s.c.x1d	–65.1 ± 8.7	–76.5 ± 10	–90.4 ± 6.7	–93.8 ± 6.9	–95.9 ± 7.1	–96.4 ± 5.9	–98.0 ± 2.3	–97.6 ± 4
DEC + tetracycline	50 mg/kg p.o.x5d + 200 mg/kg p.o.x5d	–80.4 ± 11.2	–72.2 ± 6.6	–80.3 ± 4.2	–71.7 ± 6.5	–77.5 ± 8.9	–65.7 ± 12.8	–60.6 ± 14.5	–57.4 ± 13.2
DEC + ivermectin + tetracycline	50 mg/kg p.o.x5d + 2 mg/kg s.c.x1d + 200 mg/kg p.o.x5d	–79.1 ± 11.3	–73.0 ± 13.3	–81.0 ± 6.9	–82.4 ± 4.8	–89.9 ± 3.8	–92.8 ± 5.1	–86.8 ± 7.1	–92.1 ± 2.4
DEC + albendazole	50 mg/kg p.o.x5d + 200 mg/kg p.o.x5d	–92.7 ± 5.4	–82.5 ± 8.7	–84.7 ± 6.1	–89.9 ± 4.0	–87.7 ± 5.6	–92.1 ± 2.8	–89.9 ± 7.9	–96.0 ± 3.4
DEC + albendazole + tetracycline	50 mg/kg p.o.x5d + 200 mg/kg p.o.x5d + 200 mg/kg p.o.x5d	–85.4 ± 6.8	–90.8 ± 6.7	–94.6 ± 2.1	–94.3 ± 2.7	–94.8 ± 2.2	–94.8 ± 4.0	–95.3 ± 4.6	–95.6 ± 2.6
Albendazole + tetracycline	200 mg/kg p.o.x5d + 200 mg/kg p.o.x5d	–45.3 ± 6.9	–40.2 ± 7.2	–50.3 ± 4.0	–48.7 ± 4.6	–46.7 ± 11.2	–58.9 ± 14.5	–28.8 ± 8.7	–23.0 ± 16.7
DEC + ivermectin + albendazole	50 mg/kg p.o.x5d + 2 mg/kg s.c.x1d + 200 mg/kg p.o.x5d	–72.4 ± 23.2	–75.4 ± 20.2	–84.0 ± 19.8	–88.6 ± 10.7	–98.3 ± 3.3	–96.6 ± 6.7	–99.8 ± 1.0	–100.0 ± 1.0
Ivermectin + albendazole	2 mg/kg s.c.x1d + 200 mg/kg p.o.x5d	–44.2 ± 34	–42.3 ± 22.6	–14.8 ± 36.8	–62.2 ± 15.1	–78.3 ± 6.8	–67.6 ± 19.7	–72.5 ± 4.2	–67.8 ± 13.9
Ivermectin + albendazole + tetracycline	2 mg/kg s.c.x1d + 200 mg/kg p.o.x5d + 200 mg/kg p.o.x5d	–57.7 ± 19.2	–45.5 ± 14.0	–80.9 ± 2.7	–91.1 ± 5.7	–92.4 ± 4.9	–89.5 ± 9.1	–87.9 ± 12.0	–85.9 ± 23.8
Ivermectin + tetracycline	2 mg/kg s.c.x1d + 200 mg/kg p.o.x5d	–10.0 ± 42.1	–43.1 ± 20.4	–58.8 ± 18.6	–72.1 ± 11.2	–85.3 ± 9.1	–74.2 ± 4.2	–69.1 ± 17.8	–63.2 ± 19.3
DEC	50 mg/kg p.o.x5d	–84.6 ± 13.8	–77.6 ± 19.4	–32.3 ± 42.3	–15.2 ± 56.7	+24.2 ± 135.0	+137.1 ± 125.2	+204.4 ± 123.5	+268.5 ± 102.3
Albendazole	200 mg/kg p.o.x5d	–13.2 ± 28.5	+44.0 ± 81.3	+51.7 ± 54.4	+51.5 ± 44.9	–25.4 ± 19.8	+29.5 ± 49.8	–19.5 ± 24.8	+69.0 ± 18.4
Ivermectin	2 mg/kg s.c.x1d	+34.6 ± 26.4	+10.7 ± 71.3	–9.5 ± 27.2	–51.5 ± 44.5	–56.3 ± 24.9	–29.9 ± 41.4	–48.5 ± 38.2	–36.2 ± 24.0
Tetracycline	200 mg/kg p.o.x5d	+62.3 ± 46.9	+138.4 ± 113.1	+168.4 ± 121.0	+184.5 ± 165.3	+187.5 ± 175.4	+219.7 ± 168.5	+220.4 ± 145.0	+232.4 ± 130.2
Untreated control	–	+182.5 ± 125.1	+224.5 ± 115.4	+294.0 ± 148.6	+293.2 ± 82.7	+268.9 ± 127.9	+352.8 ± 199.1	+417.3 ± 175.0	+460.5 ± 165.6

inhibit embryo-static effect of ivermectin, which could have been due to different parasite species. Our findings thus do not recommend albendazole–ivermectin combination, especially in brugian filariasis where it may interfere with the embryostatic efficacy of this potent drug. Interestingly, tetracycline brought about significant ($P < 0.05$) reversal of this impaired action.

It was interesting to observe that all the three antifilarial drugs in combination provided the best MIF efficacy by totally eliminating the circulating microfilariae from day 90 onwards with 90% suppression even on day 60 (Table 1). Animals treated with the DEC–ivermectin–albendazole combination demonstrated significant (62.6%) killing of adult worms (mostly male), sterilizing all the surviving female *B. malayi*. The present findings depicts this drug combination as the best one, by completely interrupting transmission of the disease. However, coendemicity with onchocerciasis in other parts of the world precludes the use of DEC because of severe side-effects.

Tetracycline did not promote MIF efficacy of DEC on day 8, but the duration of microfilariae (mf) suppression was significantly prolonged resulting into >60% suppression on day 90/105 in contrast to short-lived (45 days) MIF efficacy of DEC (Table 1). Ivermectin alone did not exert any microfilaricidal effect on day 15. However, co-administration with tetracycline resulted into 50% mf decline on day 15, which progressed further up to 86% on day 60 in contrast to 56% suppression by ivermectin alone (Table 1). Albendazole–tetracycline combination was moderately microfilaricidal (45%), although both drugs individually were ineffective (Table 1). Five days short-term treatment with tetracycline could not enhance the adulticidal efficacy of any antifilarial used in the present study (Figures 1 and 2).

The restricted localization of *Wolbachia* bacteria within the vacuoles in the lateral cords and reproductive organs of the parasite intracellularly, might possibly have led to the inactivity of antibiotic in a short-term treatment schedule on bacteria and in turn on adult parasites. Possibly higher concentrations of tetracycline are required for a longer time to clear the *Wolbachia* from nematode[24]. More than 40 days of treatment in rodents and 9 months treatment in cattle is recommended for achieving macrofilaricidal activity of tetracycline[25]. We have recently re-

ported improved antifilarial efficacy of liposomized tetracycline in experimental brugian filariasis with short-term treatment at a very low dose and with total depletion of *Wolbachia* from adult *B. malayi*[26]. Few studies with regard to human filariasis have recently been reported revealing significant micro and macrofilaricidal efficacy in onchocerciasis patients treated with 100 mg/kg doxycycline for six weeks[23,27]. There is growing evidence that *Wolbachia* in filariae may be important provokers of at least some of the pathology associated with these infections[24,26]. Thus antibiotic treatment, irrespective of any direct effect on the nematodes, may prove beneficial in alleviating the signs and symptoms of lymphatic filariasis. Antifilarial therapy preceded by antibiotic treatment may also prevent adverse consequences associated with the massive release of bacteria-derived proinflammatory lipopolysaccharides[24]. Further studies are in progress to observe the effect of prior depletion of *Wolbachia* on the antifilarial efficacy of standard drugs.

1. Addiss, D. G., Global disease elimination and eradication as public health strategies. Lymphatic filariasis. *Bull. WHO*, 1998, **76**, 145–146.
2. Michael, E., The population dynamics and epidemiology of lymphatic filariasis. In *Lymphatic filariasis – Tropical Medicine: Science and Practice* (ed. Nutman, T. B.), Imperial College Press, London, 2000, vol. 1, pp. 41–82.
3. Ottesen, E. A., Duke, B. O. L., Karan, M. and Bebehani, K., Strategies and tools for control/elimination of lymphatic filariasis. *Bull. WHO*, 1997, **75**, 491–503.
4. Moulia-Pelat, J. P., Glazion, P., Nguyen, I. N., Gaxotte, P. and Nicolas, I., Combination of ivermectin plus diethylcarbamazine, a new effective tool for control of lymphatic filariasis. *Trop. Med. Parasitol.*, 1995, **46**, 9–12.
5. Shenoy, R. K., George, L. M., John, A., Suma, T. K. and Kumaraswami, V., Treatment of microfilaraemia in asymptomatic brugian filariasis: The efficacy and safety of the combination of single doses of ivermectin and diethylcarbamazine. *Ann. Trop. Med. Parasitol.*, 1998, **92**, 579–585.
6. Shenoy, R. K., Dalia, S., John, A., Suma, T. K. and Kumaraswami, V., Treatment of the microfilaraemia of asymptomatic brugian filariasis with single doses of ivermectin, diethylcarbamazine or albendazole, in various combinations. *Ann. Trop. Med. Parasitol.*, 1999, **93**, 643–651.
7. Shenoy, R. K., John, A., Babu, B. S., Suma, T. K. and Kumaraswami, V., Two-year follow-up of the microfilaraemia of asymptomatic brugian filariasis, after treatment with two annual single doses of ivermectin, diethylcarbamazine and albendazole in various combinations. *Ann. Trop. Med. Parasitol.*, 2000, **94**, 607–614.
8. Ismail, M. M. *et al.*, Efficacy of single dose combination of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans. R. Soc. Trop. Med. Hyg.*, 1998, **92**, 94–97.
9. Hoerauf, A. *et al.*, Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J. Clin. Invest.*, 1999, **103**, 11–18.
10. Hoerauf, A., Volkmann, L., Hamelmann, C., Adjei, O., Autenreith, I., Fleischer, B. and Buttner, T. W., Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet*, 2000, **355**, 1242–1243.
11. Bajpai, P., Vedi, S., Owais, M., Sharma, S. K., Saxena, P. N. and Misra-Bhattacharya, S., Use of liposomized tetracycline in elimination of *Wolbachia* endobacterium of human lymphatic filariid *Brugia malayi* in rodent model. *J. Drug Target.*, 2005, **13**, 375–381.
12. Weil, G., Lammie, P. J. and Weiss, N., The ICT filariasis test: A rapid format antigen test for diagnosis of bancroftian filariasis. *Parasitol. Today*, 1997, **13**, 401–404.
13. Sanger, I., Lammer, G. and Kimming, P., Filarial infection in *Mastomys natalensis* and their relevance in experimental chemotherapy. *Acta Trop.*, 1981, **38**, 277–288.
14. Misra-Bhattacharya, S., Katiyar, D., Bajpai, P., Tripathi, R. P. and Saxena, J. K., 4-Methyl-7-(tetradecanoyl)-2H-1-benzopyran-2-one: A novel DNA topoisomerase II inhibitor with adulticidal embryo static activity against subperiodic *Brugia malayi*. *Parasitol. Res.*, 2004, **92**, 177–182.
15. Tisch, D. J., Michael, E. and Kazura, J. W., Mass chemotherapy options to control lymphatic filariasis: A systematic review. *Lancet Infect. Dis.*, 2005, **5**, 514–523.
16. Addiss, D. G. *et al.*, Randomised placebo controlled comparison of ivermectin and albendazole alone and in combination for *Wuchereria bancrofti* microfilaraemia in Haitian children. *Lancet*, 1997, **ii**, 480–484.
17. Ottesen, E. A., Ismail, M. M. and Horton, J., The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol. Today*, 1999, **15**, 382–386.
18. Bentwich, Z., Kaliskovich, A., Weisman, Z., Borkow, G., Beyers, N. and Beyers, A. D., Can eradication of helminthic infections change the face of AIDS and tuberculosis? *Immunol. Today*, 1999, **20**, 485–487.
19. Nacher, M., Malaria vaccine trials in a wormy world, *Trends Parasitol.*, 2001, **17**, 563–566.
20. Pani, S. P., Subramanyam Reddy, G., Das, L. K., Vanamail, P., Hoti, S. L., Ramesh, J. and Das, P. K., Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or coadministration of albendazole with DEC in the clearance of *Wuchereria bancrofti* in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study. *Filaria J.*, 2002, **1**(1), 1.
21. Ravindran, B., Mass drug administration to treat lymphatic filariasis. *Lancet*, 2002, **359**, 1948.
22. Kraker, de M. E., Stolk, W. A., Van Oortmarssen, G. J. and Habbema, J. D., Model-based analysis of trial data: Microfilaria and worm-productivity loss after diethylcarbamazine–albendazole or ivermectin–albendazole combination therapy against *Wuchereria bancrofti*. *Trop. Med. Int. Health*, 2006, **11**, 718–728.
23. Hoerauf, A. *et al.*, Doxycycline in the treatment of human onchocerciasis: Kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female Onchocerca worms. *Microbes Infect.*, 2003, **5**, 261–273.
24. Jayakody, R. L., de Silva, C. S. S. and Weerasinghe, W. M. T., Treatment of bancroftian filariasis with albendazole: Evaluation of efficacy and adverse reactions. *Trop. Biomed.*, 1993, **10**, 19–24.
25. Taylor, M. J., Bandi, C., Hoerauf, A. M. and Lazdins, J., *Wolbachia* bacteria of filarial nematodes: A target for control? *Parasitol. Today*, 2000, **16**, 179–180.
26. Bandi, C. A., Traseeb, J. A. and Bratting, W. N., *Wolbachia* in filarial nematodes: Evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. *Vet. Parasitol.*, 2001, **98**, 215–238.
27. Hoerauf, A., Mand, S., Adjei, O., Fleischer, B. and Buttner, D. W., Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline and microfilaridermia after ivermectin treatment. *Lancet*, 2001, **357**, 1415–1416.

ACKNOWLEDGEMENTS. Technical assistance provided by A. K. Roy and R. N. Lal is acknowledged. Financial assistance from Council of Scientific and Industrial Research, New Delhi, in the form of senior and junior research fellowships to P.B. and S.S. is acknowledged.

Received 24 June 2005; revised accepted 11 September 2006