Medical and Veterinary Entomology (1999) **13**, 120–123

Mass treatment with ivermectin for filariasis control in Papua New Guinea: impact on mosquito survival

M. J. BOCKARIE, J. L. K. HII*, N. D. E. ALEXANDER, F. BOCKARIE, H. DAGORO, J. W. KAZURA† and M. P. ALPERS

Papua New Guinea Institute of Medical Research, *School of Public Health and Tropical Medicine, James Cook University of North Queensland, and †Division of Geographic Medicine, Case Western Reserve University School of Cleveland, Ohio, U.S.A.

Abstract. Field studies were carried out to determine the impact of mass human treatment with ivermectin on the survival of anthropophagic mosquitoes of the *Anopheles punctulatus* complex (Diptera: Culicidae), the vectors of lymphatic filariasis and malaria in Papua New Guinea. In a village where mass treatment had been given, using 400 μg/kg ivermectin plus 6 mg/kg diethylcarbamazine citrate (DEC), we performed pre- and post-treatment collections of freshly blood-engorged mosquitoes from the same nine bedrooms. All blood-fed mosquitoes collected less than 4 days after mass treatment died within 9 days, whereas 67% of those collected before treatment survived for >9 days. Comparison (using the log-rank test) of the survival curves for mosquitoes collected (i) before treatment, (ii) <4 days after treatment, and (iii) 28 days after treatment, showed the survival rate of group (ii) to be significantly lower than the other two ($\chi^2 = 176$, df = 2, $P < 0.0001$). Pre- and post-treatment all-night landing catches showed no reduction in human biting rates in the experimental village. In another village, where people were mass treated with ivermectin (400 μg/kg) only, the survival rates of freshly blood-engorged *An.punctulatus* collected from bedroom resting-sites less than 1 day after treatment, were compared to similar collections carried out at the same time in a nearby village where people were not treated with ivermectin. The 48-h survival rate for the ivermectin-treated village was 31% compared to 94% for the other; this difference was highly significant ($\chi^2 = 32.42$, df = 1, $P < 0.0001$). Mosquitoes fed 2 months post-treatment with DEC or collected 38 days post-treatment with ivermectin had normal survival rates. We conclude that the duration of the systemic lethal effect of ivermectin on mosquitoes is insufficient to be of epidemiological significance in filariasis control programmes that are based on biannual and annual single-dose treatments, but might reduce vectorial capacity sufficiently to block epidemics of dengue or even malaria.

Key words. *Anopheles koliensis, An.punctulatus*, diethylcarbamazine citrate (DEC), filariasis vectors, ivermectin, lymphatic filariasis, survival rate, vector control, Papua New Guinea.

Introduction

Ivermectin belongs to a group of semi-synthetic macrocyclic lactones, the avermectins, which exhibit a high level of toxicity to a wide spectrum of arthropods including mosquitoes (Wilson, 1993). Following the introduction of ivermectin in 1987 for the treatment of human onchocerciasis, the number of annual treatments reached 9.2 million in 1993 and continues to grow rapidly (W.H.O., 1995). In many parts of Africa, onchocerciasis, which is transmitted by blackflies (Diptera: Simuliidae), occurs mainly in areas endemic for other mosquito-borne diseases such as malaria and lymphatic filariasis. Studies in French Polynesia (Cartel *et al.*, 1992) and Papua New Guinea (PNG) (Bockarie *et al.*, 1998) have

Correspondence: Dr Moses J. Bockarie, Papua New Guinea Institute of Medical Research, P.O. Box 378, Madang, Papua New Guinea. E-mail: mbockarie@datec.com.pg

shown that ivermectin, given alone or in combination with diethylcarbamazine citrate (DEC), is very effective in the treatment of Bancroftian filariasis. Thus, WHO (1997) is preparing to launch a world-wide programme to eliminate lymphatic filariasis by giving anthelminthic treatment annually to a billion infected people. In view of reports indicating reduced survival of mosquitoes (Diptera: Culicidae) fed on people treated with ivermectin (Pampiglione *et al*., 1985; Cartel *et al*., 1991), this drug could reduce the transmission of more general mosquito-borne diseases. However, no field data exist on the impact of ivermectin on mosquito survival, and the wider consequences of its effect on filaria transmission have not previously been considered.

In 1994 we started community-wide trials to determine the effect of mass drug administration (MDA) on the transmission of lymphatic filariasis in the East Sepik Province, PNG (Bockarie *et al*., 1998). Experimental studies (Bockarie *et al*. unpublished data) conducted during these trials also showed reduced survival of mosquitoes fed on people treated with DEC. These findings warranted a field evaluation of the impact of mass ivermectin treatment on mosquito survival, and this paper describes the results of community-wide studies involving wild-caught mosquitoes.

Materials and Methods

Study area

Studies were conducted in the villages of Nanaha, Warasikau and Yauatong, in the Dreikikir area (6°00′S 147°00′E) of East Sepik Province, PNG. The study area has already been described in detail by Bockarie *et al*. (1998). Human lymphatic filariasis caused by *Wuchereria bancrofti* (Cobbold) is highly endemic in the area, with anopheline mosquitoes as the main vectors (Bockarie *et al*., 1996). Prior to the present study, no MDA had commenced in Warasikau, but single-dose annual mass chemotherapy had started in 1994 using ivermectin plus DEC at Nanaha and using DEC alone at Yauatong.

Sampling and processing of mosquitoes

At Nanaha village in September 1995, fully blood-fed, indoor-resting mosquitoes were collected from the same nine houses during the mornings of the 3 days immediately preceding and the 3 days consecutively following MDA. The treatment of all the villagers was with DEC (6 mg/kg) plus ivermectin (400 µg/kg). Mosquito indoor-resting collections were repeated 28 days later in the same nine houses. Blood-engorged mosquitoes were held in cups (twenty per cup) in a cool box, in a village house and fed with sugar solution for 9 days. They were checked every 6 h to monitor survival. Dead mosquitoes were removed, counted and identified.

At Warasikau village in December 1996, following MDA with ivermectin (400 µg/kg) alone the previous night, mosquito collections were also performed in two houses. During the same morning, similar indoor-resting collections were also made in two houses at Yauatong village, where mass treatment with DEC alone had been given in October 1996. Fully blood-engorged mosquitoes collected from all four houses were held in cups inside a cool box kept in a house at Yauatong. Mosquito collections were repeated in the same four houses 38 days after mass treatment in Warasikau and blood-fed females were maintained and monitored as before. Before the collections were carried out in each house, the inhabitants were asked about their use of mosquito coils, bednets and anti-mosquito sprays.

To determine any effect of mass treatment with ivermectin on human–mosquito contact, we analysed entomological data obtained in 1994 before and after the first MDA at Nanaha in September 1994. Mosquito collections were conducted during the dry season, July–December 1994, by all-night landing catches as described by Bockarie *et al*. (1996). Hungry mosquitoes were captured between 18.00 hours and 06.00 hours as they attempted to feed on humans seated, on benches near houses, with feet and legs bared to the knee. Mosquito collectors worked in weekly rotations in four sections of the village, i.e. four nights monthly, for 6 consecutive months. Mosquitoes were identified in the field and stored separately in 70% ethanol for transport to the laboratory where they were later stained for filaria parasites using Mayer's acid haemalum (Nelson, 1958). Specimens were individually dissected and examined to assess the infection rate with *W.bancrofti* larvae of each stage.

Table 1. Survival-rates (%) for 48 h of blood-engorged *Anopheles punctulatus* mosquitoes caught resting in bedrooms in Warasikau (ivermectin treated) and Yauatong (not treated with ivermectin), in East Sepik Province, PNG.

	Days post-treatment			
	< 1		37–38	
Village	*n*	%	*n*	%
Treated (Warasikau)	45	31.1	36	97.2
Untreated (Yauatong)	35	94.3	53	100

Results

Preliminary experimental studies indicated that > 69% of *An.punctulatus* mosquitoes died within 24 h of feeding on people treated with ivermectin. Hence the monitoring of survival rates was generally limited to 48 h in later studies (Table 1). The 48-h survival rate (31%) of mosquitoes collected in Warasikau on the morning after treatment with ivermectin alone was very significantly lower than for mosquitoes collected in Yauatong (94%), where the inhabitants had been treated with DEC 2 months previously ($\chi^2 = 32.42$, $P < 0.0001$). Mosquitoes collected from the ivermectin-treated village of Warasikau 38 days post-treatment had a similar 48-h survival rate (97%) to mosquitoes collected

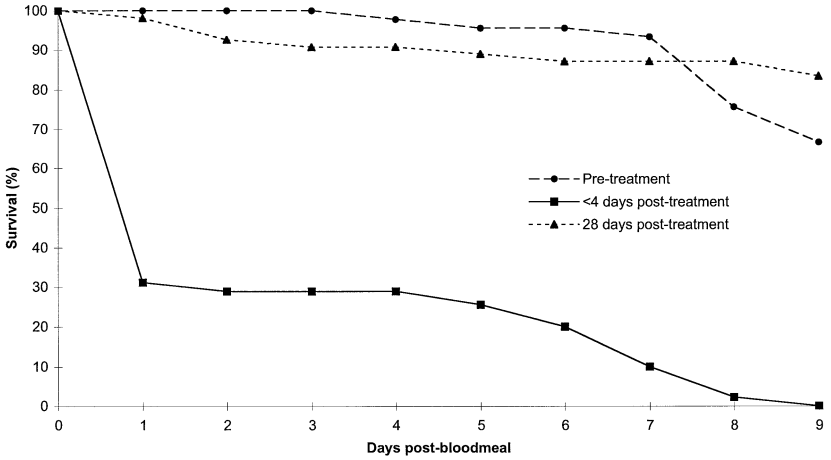

Fig. 1. Survival-rates for 9 days (cumulative percentage) of wild-caught fully blood-engorged indoor-resting *Anopheles punctulatus* mosquitoes, collected before and after mass human treatment with ivermectin (400 μg/kg) in Nanaha village, East Sepik Province, PNG.

Table 2. *Anopheles punctulatus* human biting rates (bites/person/month) and *Wuchereria bancrofti* infection rates of mosquitoes collected before and after mass human treatment with ivermectin (400 mg/kg) plus DEC (6 mg/kg) in Nanaha village, East Sepik Province, PNG.

Period	Month	Bites/person/month	L1, L2 or L3 infected % (*n*)
Pre-treatment	July	177	8.4 (166)
	August	36	15.2 (33)
	September	84	8.5 (71)
	Mean	89	9.3 (270)
Post-treatment	October	256	0 (220)
	November	90	0 (79)
	December	26	0 (24)
	Mean	124	0 (323)
	P-value	1.3×10^{-5}*	1×10^{-8}†

*ANOVA *F*-statistic=19.39.
† $\chi^2 = 31.22$, df = 1.

from Yauatong (100%), showing no long-term systemic insecticidal efficacy of either filaricidal treatment.

Figure 1 shows the cumulative mortality curves for indoor-resting blood-fed anopheline mosquitoes sampled from houses in Nanaha village before and after the mass treatment of the inhabitants with ivermectin and DEC in combination. Pre-treatment collections yielded a total of forty-five freshly blood-engorged *Anopheles* mosquitoes, comprising twenty-three *An.punctulatus* and twenty-two *An.koliensis*. Similar collections 1–3 days after treatment yielded thirty-six *An.punctulatus* and fifty-four *An.koliensis.* Collections made 28 days post-treatment yielded forty-four *An.punctulatus* and ten *An.koliensis*. Chi-square tests showed the survival rates for both species to be similar in all three groups, but different between groups ($P < 0.001$).

All mosquitoes collected at Nanaha 1–3 days post-treatment died within nine days, mostly (70%) dying within 24 h of collection, whereas 67% of those collected before treatment survived 10 days or more. For mosquitoes collected 28 days after treatment the survival rate for 10 days (83%) was higher than before treatment, but the difference was not significant ($\chi^2 = 3.71$, df = 1, $P > 0.05$). Comparison of the three survival curves (using the log-rank test) showed that mosquitoes caught < 4 days post-treatment had a significantly lower survival rate than those collected pre-treatment or 28 days post-treatment ($\chi^2 = 176$, df = 2, $P < 0.0001$). No residents of houses used for indoor-resting mosquito collections reported the use of mosquito coils, anti-mosquito sprays or other measures in their rooms during the study period.

The mean number of *An.punctulatus* complex mosquitoes caught per person per month in Nanaha was higher after ($n = 124$) than before MDA ($n = 89$) and the difference was highly significant (ANOVA *F*-statistic = 19.36, $P = 1.3 \times 10^{-5}$). Monthly filarial infection rates of the *An.punctulatus* complex ranged from 8.4% to 15.2% before treatment, but were zero in all 3 months following treatment (Table 2).

Discussion

Mass ivermectin treatment of PNG villagers resulted in dramatic reductions of survival rates for mosquitoes that fed on their blood up to 4 days after treatment. The effectiveness of ivermectin as a systemic insecticide against mosquitoes is well known (Pampiglione *et al*., 1985; Tesh & Guzman, 1990), but this is the first report of a field study involving wild-caught mosquitoes after they had fed naturally on ivermectin-treated people following their normal life style. No such effect was observed in blood-engorged mosquitoes collected 28 days or more after treatment, suggesting that the lethal effect of ivermectin was of a short duration. This is contrary to reports by Cartel *et al*. (1991), working with *Aedes polynesiensis* in French Polynesia, who observed significant reductions in

mosquito survival rates after feeding on people treated with ivermectin and DEC up to three months previously. In the present study, the 48-h survival rate of mosquitoes collected from one village was 100% two months after mass treatment with DEC, and there is no suggestion that DEC also acts as a systemic insecticide.

Failure to find filaria-infected mosquitoes (among 372 caught) during the 3 months following mass treatment in Nanaha suggests that mosquitoes had fed on people whose microfilaraemia had decreased after mass treatment. Before MDA the mosquito infection rate was over 9%. The geometric mean microfilaria density decreased by 86.6% in Nanaha after MDA once with DEC plus ivermectin (Bockarie *et al*., 1998).

The vectors *Anopheles punctulatus* and *An.koliensis* are highly anthropophagic (Charlwood *et al*., 1986) and, in the absence of domestic pigs, as was the case in Nanaha during 1994, they feed almost exclusively on humans. Previous studies in nearby villages of East Sepik Province showed human blood indices of 90% and 79% in *An.punctulatus* and *An.koliensis*, respectively (Hii *et al*., 1997). Although the ivermectin dose used in the present study was four times the maximum of 100 µg/kg used in the French Polynesian study, human biting rates actually rose in the month following mass treatment, indicating that the overall population impact of the systemic ivermectin on mosquito survival and reproduction was < 30 days. Likewise, for onchocerciasis transmission in West Africa, monthly biting rates of *Simulium* blackflies were not altered following treatment of humans with ivermectin (Trpis *et al*., 1990). Moreover, Mahon *et al*. (1993) reported that ivermectin-treated sheep produced dung that was acutely toxic to *Lucilia cuprina* blowflies for only a short period, restricted to the first day post-treatment. In Papua New Guinea, where *An.punctulatus* is the principal vector of both malaria and lymphatic filariasis, mass treatment with ivermectin gave significant short-term reductions in the transmission rates of *W.bancrofti* (Bockarie *et al*., 1998).

Whereas the systemic lethal effect of ivermectin on mosquitoes is too transient to be of much epidemiological significance in a filariasis control programme based on biannual or annual single-dose treatments, it is possible that epidemics of other mosquito-borne diseases such as dengue (or even malaria) could be blocked by timely ivermectin treatment of the people at risk (and their livestock) on whom the vector populations depend for bloodmeals.

Acknowledgements

Our study of ivermectin effects on mosquito survival was funded by a grant from the World Health Organization Western Pacific Region Office (W.H.O./W.P.R.O.). Clearance for this study was given by the Medical Research Advisory Committee of Papua New Guinea.

References

Bockarie, M., Alexander, N.D.E., Hyun, P., Dimber, Z., Bockarie, F., Ibam, E., Alpers, M.P. & Kazura, J.W. (1998) Randomised community-based trial of annual single-dose diethylcarbamazine with or without ivermectin against *Wuchereria bancrofti* infection in human beings and mosquitoes. *Lancet*, **351**, 162–168.

Bockarie, M., Kazura, J., Alexander, N., Dagoro, H., Bockarie, F., Perry, R. & Alpers, M. (1996) Transmission dynamics of *Wuchereria bancrofti* in East Sepik Province, Papua New Guinea. *American Journal of Tropical Medicine and Hygiene*, **54**, 577–581.

Cartel, J.-L., Nguyen, J.L., Moulia-Pelat, J.-P., Plichart, R., Martin, P.M.V. & Spiegel, A. (1992) Mass chemotherapy of lymphatic filariasis with a single dose of ivermectin in a Polynesian community with high *Wuchereria bancrofti* infection rate. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 537–540.

Cartel, J.-L., Sechan, Y., Spiegel, A., Nguyen, L., Barbazan, P., Martin, P.M.V. & Roux, J.F. (1991) Cumulative mortality rates in *Aedes polynesiensis* after feeding on polynesian *Wuchereria bancrofti* carriers treated with single dose of ivermectin, diethylcarbamazine and placebo. *Tropical Medicine and Parasitology*, **42**, 343–345.

Charlwood, J.D., Graves, P.M. & Alpers, M.P. (1986) The ecology of the *Anopheles punctulatus* group of mosquitoes from Papua New Guinea. *Papua New Guinea Medical Journal*, **29**, 19–26.

Hii, J.K.L., Smith, T., Mai, A., Mellor, S., Lewis, D., Alexander, N. & Alpers, M.P. (1997) Spatial and temporal variation in abundance of *Anopheles* (Diptera: Culicidae) in malaria endemic area in Papua New Guinea. *Journal of Medical Entomology*, **34**, 193–205.

Mahon, R.J., Wardhaugh, K.G., van Gerwen, A.C.M. & Whitby, W.A. (1993) Reproductive development and survival of *Lucilia cuprina* Wiedemann when fed sheep dung containing ivermectin. *Veterinary Parasitology*, **48**, 193–204.

Nelson, G.S. (1958) Staining of filarial larvae in insects before dissection. *Bulletin of the World Health Organization*, **19**, 204.

Pampiglione, S., Majori, G., Petrangeli, G. & Romi, R. (1985) Avermectins, MK-933 and MK-936, for mosquito control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 797–799.

Tesh, R.B. & Guzman, H. (1990) Mortality and infertility in adult mosquitoes after the ingestion of blood containing ivermectin. *American Journal of Tropical Medicine and Hygiene*, **43**, 229–233.

Trpis, M., Childs, J.E., Fryauff, D.J., Green, B.M., Williams, P.N., Munoz, B.E., Pacque, M.C. & Taylor, H.R. (1990) Effect of mass treatment of a human population with ivermectin on transmission of *Onchocerca volvulus* by *Simulium yahense* in Liberia, West Africa. *American Journal of Tropical Medicine and Hygiene*, **42**, 148–156.

W.H.O. (1995) *Onchocerciasis and its control: report of the WHO Expert Committee on Onchocerciasis Control*. World Health Organization, Geneva. Technical Report Series No. 852.

W.H.O. (1997). *Lymphatic filariasis: Reasons for hope*. Document WHO/CTD/Fil/97.4 Rev.1.

Wilson, M.L. (1993) Avermectins in arthropod vector management – prospects and pitfalls. *Parasitology Today*, **9**, 83–87.

Accepted 12 September 1998