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FIELD TRIALS OF BIOLARVICIDE *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* STRAIN 164 AND THE LARVIVOROUS FISH *APLOCHEILUS BLOCKI* AGAINST *ANOPHELES STEPHENSI* FOR MALARIA CONTROL IN GOA, INDIA

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ABSTRACT. Severe outbreaks of malaria occurred in the coastal villages of the Candolim Primary Health Centre (PHC) of Goa, India, in 1993 and 1994. These outbreaks were associated with accelerated construction activity with an influx of migrant laborers. The weekly application of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) strain 164 at 1 g/m^2 and introduction of the indigenous larvivorous fish *Aplocheilus blocki* in major breeding habitats of *Anopheles stephensi* replaced ongoing DDT spraying and pyrethrum fogging in June 1994. The objective of this study was to evaluate the effects of *B.t.i.* and larvivorous fish on *An. stephensi* and subsequent transmission of malaria in the Candolim PHC, Goa, India. In 1995 the populations of *An. stephensi* in larger habitats (habitats with immatures: $t = 5.19$, $P = 0.0017$; immature density: $t = 3.57$, $P = 0.007$) and smaller habitats (habitats with immature: $t = 3.86$, $P = 0.005$; immature density: $t = 4.93$, $P = 0.002$) and malaria incidence declined substantially (malaria cases: $\chi^2 = 712$, $P < 0.001$; slide positivity rate: $\chi^2 = 10.36$, $P < 0.001$; annual parasite index; $\chi^2 = 15.1$, $P < 0.001$), whereas the incidence of malaria continued to increase in other nearby towns.

KEY WORDS *Bacillus thuringiensis* var. *israelensis, Anopheles stephensi,* malaria transmission, larvivorous fish, malaria control, vector control, field trials, biolarvicides, biological control

INTRODUCTION

At the time of launching of the National Malaria Eradication Programme (NMEP) in India in 1953, urban malaria was not recognized as a major problem and local control was left to municipalities (Sharma 1996). Urban expansion, which brought with it an increased supply of tapped water and storage tanks and cisterns, has facilitated the spread of malaria transmitted by *Anopheles stephensi* (Liston), which has become a serious problem in many towns. Annual incidence of malaria in India reported by the NMEP since the mid-1980s varied from 2 to 3 million cases. Urban malaria now contributes up to 15% of reported cases. This situation is gradually worsening, with outbreaks reported from formerly nonendemic cities.

In Goa, India, a severe outbreak of malaria occurred in Panaji in 1986. Major causes of this outbreak included increased construction activity to meet the demand of the tourist industry and housing complexes (Narasimham and Khamre 1987). As a result, many villages and towns of Goa, particularly along the coast, have been completely transformed. In an earlier study, extensive breeding of *An. stephensi* in multistory construction complexes and aggregation of migratory laborers, who serve as source of plasmodial infection, were considered as major causes of malaria outbreaks in the state of Goa (Kumar et al. 1991). A high degree of resistance to DDT in *An. stephensi* populations in Goa has been reported (Thavaselvam et al. 1993). *Anopheles stephensi* breeds in a variety of habitats in urban and suburban conditions and is not easily amenable to control (Kalra 1991). Moreover, the *An. stephensi* type form is a very efficient vector and can transmit malaria at low densities. Therefore, meticulous and well-supervised field operations, in addition to effective control tools are essential to have an effect on malaria transmitted by this species. Earlier, small-scale field trials of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) strain 164 showed good control of *An. stephensi* in construction sites in Goa, India (Kumar et al. 1995).

In 1993, a malaria outbreak occurred in formerly endemic villages of the coastal Candolim Primary Centre (PHC), Goa. This outbreak further intensified in 1994. Conventional methods of control (DDT spraying and pyrethrum fogging) proved inadequate and alternate control strategies were required. A study to evaluate *B.t.i.* strain 164 in small larval mosquito developmental sites was established. Larger developmental sites were evaluated using the indigenous larvivorous fish *Aplocheilus blocki* (Arnold). Both agents were evaluated for their impact on the *An. stephensi* population and subsequent malaria transmission.

MATERIALS AND METHODS

Study area: The study was conducted around the malaria-endemic Candolim PHC, Goa, India (population: 47,109), situated along the coast of the Arabian Sea at 15°50′N, 73°52′E. Ten villages are in this Center's jurisdiction. Two large villages

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(Candolim and Calangute; combined population: 24,252) are located along beaches that attract about half a million tourists annually. Maximum and minimum temperature fluctuated from 28.6 to 33.4°C and 19.6 to 26.7°C, respectively, in 1994 and from 28.3 to 33.8°C and 19.6 to 27.5°C, in 1995. The normal rainfall is 2,500 mm and usually occurs from April–May to October–November. In 1994, 2,894 mm of rainfall occurred during 135 days, whereas in 1995, 3,549 mm of rainfall occurred during 127 days. Average relative humidity fluctuated from 75 to 94% during both years.

Larval developmental habitats: House-to-house visits were made in the experimental area in order to locate all permanent developmental sites of *An. stephensi.* The major sites present were wells, curing waters (intentional stagnation of water on newly cast cement slabs for strengthening purpose), masonry tanks, swimming pools, ornamental fountains, rainwater pools, sunshades above windows (lintels), roof gutters, overhead tanks, sumps, and barrels.

Larvicide application: Those larval developmental sites unsuitable for the introduction of fish (i.e., masonry tanks, sump tanks, curing water on recently cast concrete, freshly constructed overhead tanks, roof gutters, rainwater pools on terraces) were treated with *B.t.i.* strain 164 serotype H-14 (trade name Bacticide) at 1 g/m^2 surface area with knapsack sprayers (16-liter capacity) equipped with flat fan nozzles. Initially, *B.t.i.* was applied as blanket spray irrespective of the presence or absence of *An. stephensi* in the habitats. However, in subsequent weeks only those habitats with larvae (detected during weekly vector surveillance) were treated. The *B.t.i.* was manufactured by the Berdsk Plant, Berdsk, Novosibirsk, Russia, and supplied through the courtesy of the Ministry of Health and Family Welfare, New Delhi, India. The *B.t.i.* formulation was homogenous dry powder (particle size range 90–500 μm). The median lethal dose value against *Aedes aegypti* L. larvae was 0.12–0.15 mg/liter. Insecticidal potency was 993 + 90 IU/mg, titrated against IPS-82. The powder was gently mixed in water to obtain a paste before gradually homogenizing it in the required quantity of water. To obtain the desired dose of 1 g/m^2, surface area the nozzle discharge, walking speed, and swath were taken into consideration and concentration was determined as follows (Bindra and Singh 1977, Matthews 1979):

application rate

= (volume of discharge from pump/minute)

÷ [width of swath

× normal walking speed (m/min)]

concentration (%)

$$= \frac{\text{desired dose (g/m}^2\text{)}}{\text{application rate (ml/m}^2\text{)}} \times 100.$$

Fish release: Aplocheilus blocki were collected from backwaters, ponds, pit wells, and fallow fields and introduced into wells, masonry tanks (a few with deep water), swimming pools, and ornamental fountains at a rate of 5 fish/m^2 surface area where necessary. Before the commencement of operations with *B.t.i.* and larvivorous fish in June 1994, all the chemical control measures (DDT spraying and thermal fogging with pyrethrum) were withdrawn from the experimental area by state health services.

Larval surveillance: To determine larval density of *An. stephensi,* habitats were sampled weekly using galvanized buckets (5-liter capacity) in wells and 300-ml bowls in other sites. Four samples were drawn from the periphery and corners and one sample was drawn from the center. Larvae were counted and classified by instar. Collection samples were reared in the laboratory and emerged adults were identified with the taxonomic keys of Christopher (1933). Dead larvae were identified following the taxonomic keys of Puri (1953).

Malaria detection and treatment: Both active and passive malaria detection and treatment facilities were provided by Candolim PHC and a special clinic opened with the help of the Malaria Research Centre at Calangute, a subcenter of the Candolim PHC in the middle of 1994. Thick and thin blood smears were stained with Jaswant Singh Bhattacharji (JSB) stain and examined under a compound microscope. Treatment of malaria patients was provided using the drug policy of NMEP of India. The parasitologic data for adjoining towns of Panaji and Porvorim were obtained from the Directorate of Health Services, Goa.

Statistical analysis: Student's *t*-test and the chi-square test for differences between paired means were applied for the statistical analysis of results of this study.

RESULTS AND DISCUSSION

Table 1, shows that 586 cases of malaria including 9 *Plasmodium falciparum* cases were reported in 1993 with a 10.6% slide positivity rate (SPR) for malaria parasites and an annual parasite index (API) of 12.4. The incidence significantly increased in 1994 with the reporting of 1,431 malaria cases (76 *P. falciparum*) with an SPR of 17.3% and an API of 30.4. In 1995, malaria incidence declined, with 263 cases reported including only 6 *P. falciparum* cases with an SPR of 7.41% and an API of 5.58. In 1993, 1994, and 1995, the annual blood examination rate (ABER) was 10.6, 17.3, and 7.5%, respectively.

The survey for possible *An. stephensi* larval developmental habitats revealed 1,195 wells, 125 masonry tanks, 107 water sumps, 100 underground tanks, 27 swimming pools, 474 overhead tanks, 259 barrels, and 4 ornamental fountains, and innumerable curing waters and rainwater pools in construction sites.

Table 1. Malaria incidence in Candolim Primary Health Centre, Goa, India, between 1993 and 1995.[1]

Year	BSE	Pos.	Pv	Pf	ABER (%)	SPR (%)	Pf (%)	SfR (%)	API
1993	5,518	586	577	9	11.7	10.6	1.53	0.16	12.44
1994	8,250	1,431	1,355	76	17.5	17.34	5.3	0.92	30.37
1995	3,547	263	257	6	7.52	7.41	2.33	0.16	5.58

[1] BSE, blood slide examined; Pos., positive for malaria; Pv, *Plasmodium vivax*; Pf, *Plasmodium falciparum*; ABER, annual blood examination rate; SPR, slide positivity rate; SfR, slide falciparum rate; API, annual parasite index.

Impact of fish introduction

A total of 54,679 *A. blocki* were introduced into 2,556 wells (some wells required reintroduction of fish): 3,428 were introduced into 173 groundwater tanks, and 3,100 were placed in 7 swimming pools and ornamental fountains. In 1994 the percentage of habitats with populations of immature *An. stephensi* ranged from 1.2% in December to 13.5% in March, with an average of 4.18% (Fig. 1). The highest larval density (16.2 per dip) in these habitats was observed during the pretreatment period in January 1994. From February to June 1994, the percentage of habitats with *An. stephensi* larvae gradually declined to 2.83% (2.06 per dip).

During 1995, the mean percentage of habitats positive and per dip density declined to 0.96% and 0.65 per dip, respectively. The percentage of habitats with immatures ranged from a low of 0.08% in December to a peak of 2.06% in June. Per dip density ranged from 0.22 in September to 1.24 in March. In general the densities of 3rd- and 4th-instar larvae and pupae were much lower in 1995 compared with 1994. Pupae were encountered in low numbers in a few habitats during the monsoon season from July to September 1995.

Significantly more habitats ($t = 2.38$, $P = 0.018$) contained *An. stephensi* immatures (as well as more mosquitoes per dip; $t = 2.9$, $P = 0.0168$) during the pretreatment period than in the posttreatment period. However, a significantly greater number of habitats contained immature mosquitoes ($t = 5.19$, $P = 0.0017$) from July to December 1994, as well as greater average number per dip ($t = 3.57$, $P = 0.007$), when compared with similar months in 1995.

Bacillus thuringiensis var. *israelensis* efficacy trials

The percentage of habitats with *An. stephensi* immatures present in samples during pretreatment in 1994 ranged from 0.82 to 3.92%, with an average of between 1.57 and 12.85 immature per dip (Fig. 2). A significant decline in number of habitats with immature mosquitoes ($t = 2.91$, $P = 0.0166$), as well as a decline in immature density ($t = 3.57$, $P = 0.007$) was observed when these data were compared with the corresponding posttreatment period in 1995. Throughout 1995, the percentage of habitats with *An. stephensi* remained low (range 0.03–0.62%). A slight increase occurred in the number of habitats with immature mosquitoes during the monsoon months (from May to August) compared with rest of 1995. However, the most pronounced

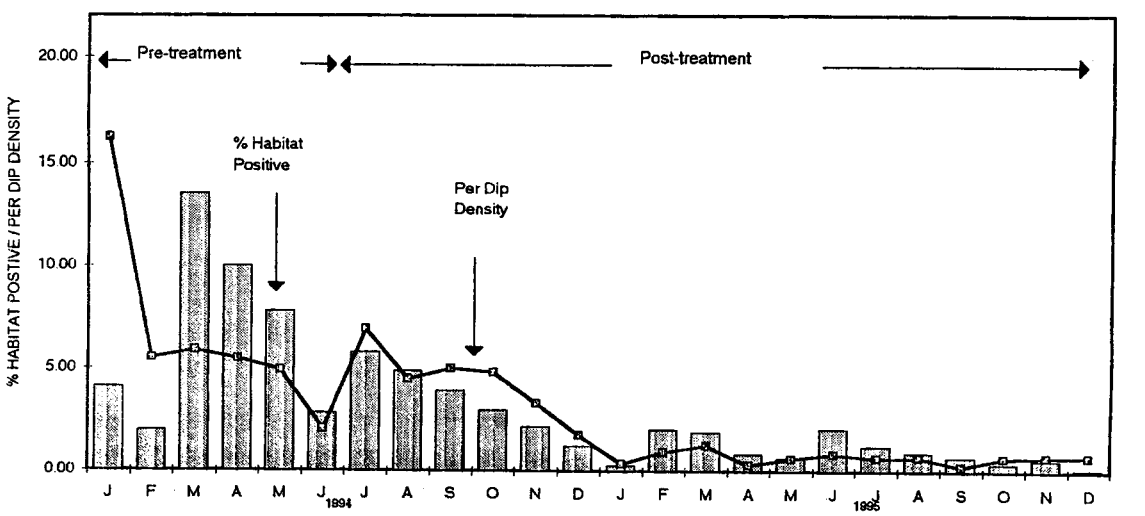

Fig. 1. Impact of introduction of *Aplocheilus blocki* on the population of immature *Anopheles stephensi*.

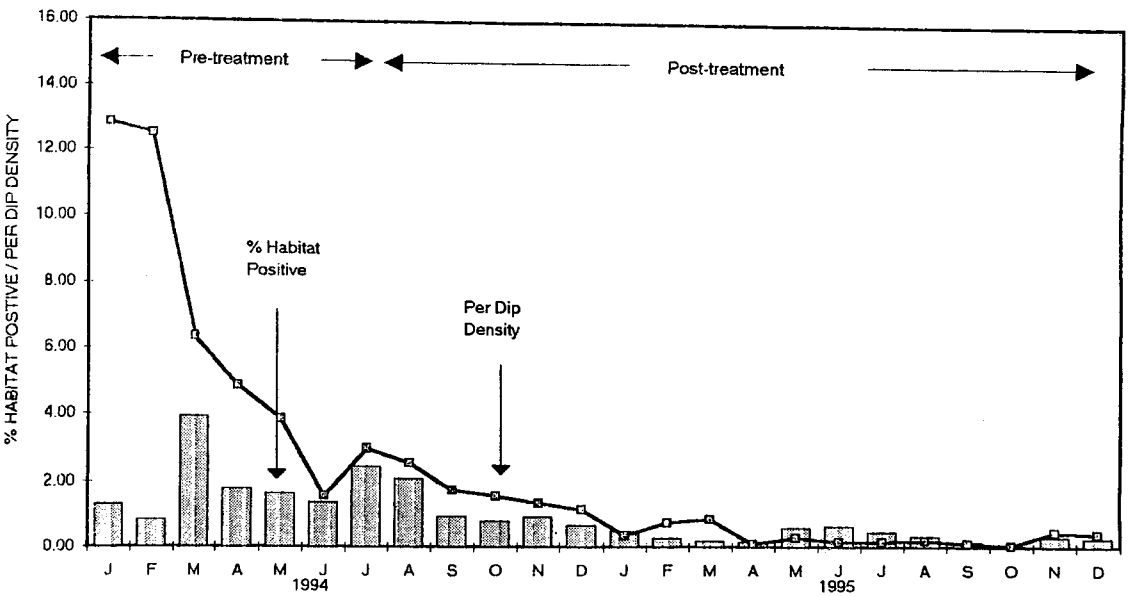

Fig. 2. Impact of weekly spraying of *Bacillus thuringiensis* var. *israelensis* at 1 g/m² in construction sites.

decrease in number of habitats containing *An. stephensi* and a subsequent decrease in number of immatures collected occurred from July to December 1995 (t = 3.86, P = 0.005; t = 4.93, P = 0.002, respectively) compared with the corresponding period of 1994.

Impact on malaria transmission

Table 2 shows that of the 3,547 slides collected from fever cases in 1995 (ABER: 14.6%), 263 were positive for malaria, including 257 *Plasmodium vivax* and 6 *P. falciparum* cases with an SPR of 7.4% and a PI of 5.6. When incidence of malaria from the pretreatment period in 1994 (i.e., from January to June) was compared with that of 1995, a reduction of 396 cases (χ^2 = 264, P < 0.001) occurred, whereas the SPR declined by 6.83%. In the peak transmission period during the monsoon season (i.e., from July to September) and during the postmonsoon phase, when malaria transmission gradually recedes in this area, the differences in the number of malaria cases (χ^2 = 712, P < 0.001), SPR (χ^2 = 10.36, P < 0.001), and parasite index (χ^2 = 15.1, P < 0.001) between 1994 and 1995 were highly significant.

Table 2. Parasitologic data of Candolim Primary Health Centre, Goa, India, 1995.[1]

Month	BSE	Pos.	Pv	Pf	SPR	SfR	PI
Jan.	432	75	74	1	17.36	0.26	1.59
Feb.	299	52	49	3	17.39	1	1.1
March	314	35	35	0	11.15	0	0.74
April	159	11	11	0	6.91	0	0.23
May	262	11	11	0	4.19	0	0.23
June	291	14	12	2	4.81	0.68	0.29
July	457	14	14	0	3.06	0	0.29
Aug.	363	12	12	0	3.3	0	0.25
Sept.	310	7	7	0	2.25	0	0.14
Oct.	192	7	7	0	3.64	0	0.14
Nov.	273	16	16	0	5.86	0	0.33
Dec.	195	9	9	0	4.61	0	0.19
Total	3,547	263	257	6	7.41	0.16	5.58

[1] BSE, blood slide examined; Pos., positive for malaria; Pv, *Plasmodium vivax*; Pf, *Plasmodium falciparum*; SPR, slide positivity rate; SfR, slide falciparum rate; PI, parasite index.

Figure 3 also shows that from April 1995 onwards, malaria transmission was impacted, as incidence in the Candolim PHC sharply declined and remained low (range 7–16 cases, SPR 2.25–6.91%, PI 0.14–0.33). It is noteworthy that not a single case of *P. falciparum* malaria was encountered in the experimental area after February 1995 except in June when 2 *P. falciparum* cases were detected from among a group of migrant laborers 2 days after their arrival in the experimental area (Table 2). The proportion of *P. falciparum* cases, which was 5.3% in 1994, was reduced to 2.3% in 1995. Moreover, the total of 6 cases of *P. falciparum* malaria in 1995 was less than 9 cases reported in 1993. History sheets of malaria patients revealed that out of total 263 cases reported in 1995, 46 (13.9%) were suspected relapse cases of *P. vivax* with previous episodes of infections either in 1994 or 1995. *Plasmodium vivax* cases with a history before 1994 were excluded from the suspected relapse cases category.

A comparison of malaria incidence in the experimental area with that in surrounding towns revealed that as a result of intervention measures,

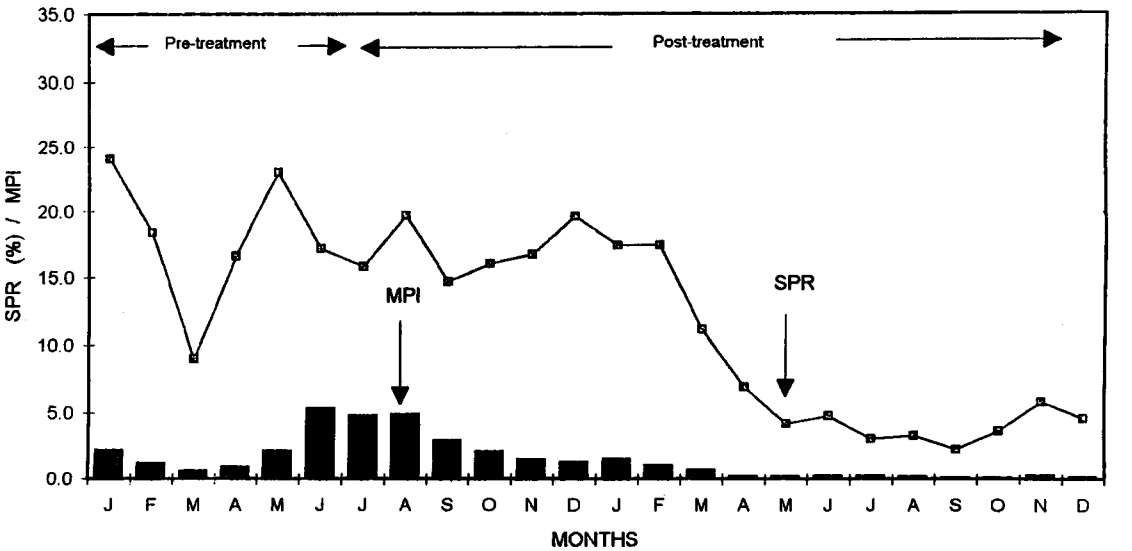

Fig. 3. Impact of intervention measures on the incidence of malaria in Candolim Primary Health Center in 1995 as compared to 1994.

the SPR, slide falciparum rate, and PI in the experimental villages of the Candolim PHC were reduced 57.3%, 82.6%, and 81.6%, respectively, in 1995. Incidence of malaria in 2 nearby malaria endemic towns (Porvorim and Panaji) increased substantially during 1995 compared with 1994 (Table 3).

Our results demonstrated the usefulness of the biolarvicide *B.t.i.* 164 (Bacticide) in combination with the indigenous larvivorous fish *A. blocki* for control of *An. stephensi* in suburban areas of Candolim PHC in Goa, monitored since July 1994. Use of *B.t.i.* and *A. blocki* led to the containment of the vector and arrested and reversed the upward trend of malaria in 1995. In an earlier study, Kumar et al. (1994) demonstrated control of malaria utilizing *Bacillus sphaericus* against *An. stephensi* in Panaji, Goa, India. In the present study, the decrease in the incidence of malaria in Candolim PHC in 1995, as evident from the data, and the near elimination of *P. falciparum* malaria from February 1995 onwards suggest that intervention measures had successfully curtailed transmission of malaria in the experimental area.

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Table 3. Comparison of malaria incidence in Candolim Primary Health Centre, Goa, India, and surrounding areas in 1994 and 1995.[1]

	1994			1995			% variation	
Area	Cases	SPR (%)	API	Cases	SPR (%)	API	API	SPR
Candolim	1,431	17.3	30.4	263	7.4	5.6	81.6 ↓	57.2 ↓
Panaji	819	10.0	19.01	2,225	21.2	51.33	170.0 ↑	112.0 ↑
Porvorim	397	26.36	22.62	928	27.1	52.9	133.86 ↑	2.81 ↑

[1] SPR, slide positivity rate; API, annual parasite index.

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