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**Short Note**



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# Availability and Utility of Local Fishes of Shahjahanpur for Mosquito Control

S. HAQ*, H. PRASAD*, R.N. PRASAD* and T. SHARMA*

A survey of fishes in Shahjahanpur in different aquatic habitats revealed 35 indigenous fish species. Out of 35 fish species, 24 were found feeding on mosquito larvae of which 6 species, viz. *Chela bacaila, Puntius stigma, Rasbora daniconius, Esomus danricus, Colisa fasciatus* and *Danio* sp., had good larvivorous potential. Most of the fish species preferred to feed on III and IV instar larvae.

In the presence of planktonic food, the consumption capacity of fishes for mosquito larvae was lesser on third day ($D_3$) of observation than on first day ($D_1$). The difference in the consumption of mosquito larvae between $D_1$ and $D_3$ was significant ($P< 0.01$). Similarly, difference in the feeding capacity of fishes in the months of September and January was highly significant ($P< 0.001$). But there was no seasonal variation in the preference of instar-wise consumption. Indigenous fish species such as *C. fasciatus, E. danricus, P. stigma, R. daniconius* and *Danio* sp. could therefore play a significant role in controlling mosquito breeding in this area.

**Key words:** Biological control, Larvivorous fishes, Mosquito control

## INTRODUCTION

Considering the insecticide resistance in mosquitoes, drug resistance in malaria parasite and the problem of environmental contamination, Malaria Research Centre launched the bioenvironmental control strategy for the control of malaria in Gujarat in 1983[1]. Similar programmes in other geoepidemiological zones of the country were also initiated to test the feasibility of this programme. Bioenvironmental control strat-

* Malaria Research Centre (Field Station), Khirni Bagh, Sadar Bazar, Shahjahanpur-242 001, India.

egy emphasises the use of larvivorous fishes to control mosquito breeding. After the introduction in India of exotic larvivorous fishes, viz. *Poecilia reticulata* and *Gambusia affinis* in 1908 and 1928 respectively[2], fishes were extensively used in mosquito control, mainly in urban areas. The evaluation of indigenous larvivores has received a new impetus with the recent accent given to the integrated vector control concept by the World Health Organization[3]. Therefore, in order to find larvivorous fishes of Indian fauna and their suitability in the control of mosquito breeding under local situations, a study was undertaken to search local fishes of Shahjahanpur district. The results of the study are reported in this paper.

## MATERIALS AND METHODS

### Survey of local fishes and their abundance

A survey of fishes in different aquatic habitats was undertaken in Shahjahanpur district (U.P.) from October 1986 to November 1987. Fishes were captured in early hours of the day from rivers, canals, ponds, drains and weed-infested water bodies using a seine net (20 × 2 m size). In lotic waters, such as rivers and drains, random nettings were carried out at 10-20 different s pots within of 5 km. In lentic waters, such as ponds, pools and ditches situated in different villages, fishes were collected through repeated nettings. Fishes were categorised as abundant, common and rare in lotic and lentic aquatic habitats on the basis of their frequency of occurrence in collections. If a particular fish was encountered in more than 50% nettings it was categorised as abundant and if encountered in 6 to 50% nettings it was categorised as common. The occurrence frequency of rare fish species was restricted to 5% nettings or less.

### Larvivorous potential test

Fishes collected from the field were brought to the laboratory and were held in tap water for two hours so that they may get acclimatised to the laboratory. Five replicates of each fish species were kept in a container of 0.45 m diam with 5 litres of tap water. In each container, 75 larvae each of I, II, III, and IV instar were used for larvivoracity test. After 24 h, i.e. on $D_1$, larval consumption was recorded. All the experiments were carried out without adding any food for fishes except mosquito larvae. The fishes were graded according to their capacity to devour mosquito larvae. Fish consuming 100 or more larvae in a day was considered as good larvivorous fish and was graded as four plus (++++); fish consuming more than 75 and up to 100 larvae as three plus (+++); those consuming >50 but <75 larvae as two plus (++); and those consuming >25 and ≤ 50 larvae as one plus (+).

### Variation in feeding capacity of fishes in different periods

During 1990-91 some important and abundantly available indigenous larvivorous fishes such as *Chela bacaila* (5-5.5 cm), *Puntius stigma* (5-6 cm), *Rasbora daniconius* (4.5-5.5 cm), *Esomus danricus* (4.5-5 cm), *Colisa fasciatus,* (4-5 cm) and *Danio* sp. (2.5-3.5 cm) were tested for feeding capacity during active feeding period in September 1990 and lean feeding period in January 1991

in the presence and absence of natural planktonic food.

Wild field-collected fishes were brought to laboratory and kept in fresh water for 2 h for acclimatisation. Experiments were conducted in two sets with 5 replicates of each fish species. In one set of experiment, fishes were kept in 5 containers of 0.45 m diam with 5 litres of tap water and 75 mosquito larvae each of I, II, III and IV instars. No fish food was supplied to the fishes in this experiment. In the other set, the experiment was conducted as first set except that fish food was supplied to the fishes in the form of natural plankton @ 1 ml/litre of water, which was collected from the pond water with the help of fine nylon mesh cloth (76 μm pore size). After 24 h instar-wise consumption of mosquito larvae was recorded. The experiment was extended for two days and observations were taken twice at intervals of 24 h, i.e. on $D_2$ and $D_3$. The data was analysed statistically.

## RESULTS

Fish survey of different aquatic habitats of Shahjahanpur district revealed 35 indigenous fish species. Of these, *C. fasciatus, E. danricus, A. mola, C. bacaila, Puntius* spp. and *R. daniconius* were found abundant in both the lotic and lentic aquatic habitats. The larvivorous potential and availability of local fishes of Shahjahanpur district are given in Table 1.

Out of 35 species, four, viz. *C. fasciatus* (++++), *E. danricus* (+++), *C. bacaila* (+++), and *Danio* sp. (+++), were found to be good larvivorous fish. Fish species such as *N. notopterus, Puntius* spp., *W. attu, M. vittatus, A. panchax, Channa* spp. *R. daniconius, C. betracus* and *A. testudinus* also showed larvivorous potential but of lesser grades only. However, other species showed little or no interest in feeding on mosquito larvae.

The results of experiments on the consumtion of mosquito larvae and variation in the feeding capacity of some of the important and abundantly available indigenous larvivorous fishes during two different periods in the presence and absence of natural food are summarised in Table 2. The larval consumption rate of fishes in September in the absence of natural food after 24 h ($D_1$) was 91.00±.6.60 for *E. danricus;* 67.00±5.34 for *C. bacaila*; 93.00±7.99 for *C. fasciatus;* 95.00± 3.27 for *Danio* sp., 64.00±6.30 for *P. stigma*; and 61.00±6.90 for *R. daniconius*. The difference in the consumption rate of fishes between $D_1$ and $D_3$ was significant ($P<0.01$). *Danio* sp. and *E. danricus* exhibited preference towards the feeding of I and II instar mosquito larvae while the other fish species consumed more III and IV instar larvae (Table 3). In the presence of planktonic food the consumption capacity of fishes for mosquito larvae decreased. Hence the difference between $D_1$ and $D_3$ was highly significant ($P<0.001$).

In January the feeding capacity of all the fish species on mosquito larvae declined. But there was no effect on seasonal variation in the preference of instar-wise consumption by the fishes. The difference in the feeding capacity of fishes on mosquito larvae between $D_1$ and $D_3$ was also significant ($P<0.05$) in the presence and absence of

**Table 1. Larvivorous potential and availability of local fishes of District Shahjahanpur**

| S. No. | Fish species | Larvivorous potential* | Availability | |
|---|---|---|---|---|
| | | | Lotic water | Lentic water |
| 1. | *Colisa fasciatus* | ++++ | Abundant | Common |
| 2. | *Chela bacaila* | +++ | Abundant | Abundant |
| 3. | *Danio* spp. | +++ | Common | Common |
| 4. | *Esomus danricus* | +++ | Abundant | Abundant |
| 5. | *Anabas testudinus* | ++ | Rare | Rare |
| 6. | *Aplocheilus panchax* | ++ | Rare | Rare |
| 7. | *Channa punctatus* | ++ | Common | Rare |
| 8. | *Chanda nama* | ++ | Abundant | Abundant |
| 9. | *Chanda ranga* | ++ | Abundant | Abundant |
| 10. | *Clarias batracus* | ++ | Rare | Rare |
| 11. | *Mystus vittatus* | ++ | Common | Common |
| 12. | *Notopterus notopterus* | ++ | Rare | Rare |
| 13. | *Puntius stigma* | ++ | Abundant | Common |
| 14. | *Puntius sophore* | ++ | Common | Common |
| 15. | *Puntius ticto* | ++ | Abundant | Common |
| 16. | *Rasbora daniconius* | ++ | Abundant | Abundant |
| 17. | *Wallago attu* | ++ | Common | Common |
| 18. | *Amblypharyngodon mola* | + | Abundant | Abundant |
| 19. | *Channa gachua* | + | Rare | Rare |
| 20. | *Channa marulus* | + | Rare | Common |
| 21. | *Chela labuca* | + | Rare | Common |
| 22. | *Cirrhinus reba* | + | Rare | Rare |
| 23. | *Mystus seenghala* | + | Rare | Common |
| 24. | *Nandus nandus* | + | Rare | Rare |
| 25. | *Puntius sarana* | + | Rare | Rare |
| 26. | *Catla catla* | — | Common | Common |
| 27. | *Channa striatus* | — | Rare | Common |
| 28. | *Cirrhinus mrigala* | — | Common | Common |
| 29. | *Heteropneustes fossilis* | — | Common | Rare |
| 30. | *Labeo rohita* | — | Common | Common |
| 31. | *Labeo bata* | — | Rare | Rare |
| 32. | *Lepidocephalichthys guntea* | — | Rare | Rare |
| 33. | *Mastacembelus armatus* | — | Rare | Rare |
| 34. | *Mastacembelus pancalus* | — | Rare | Rare |
| 35. | *Xenentodon cancila* | — | Rare | Common |

* Consumption of larvae/day : ++++ = 100 or more; +++ = 75-99; ++ = 50-74; and + = 25-49.

**Table 2. Evaluation of larvivorous potential of indigenous fishes**

| S. No. | Fish species | Size of fish in cm (range) | Active feeding period (Sep 1990) | | | | Lean feeding period (Jan 1991) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Without food | | With food | | Without food | | With food | |
| | | | $D_1$ | $D_3$ | $D_1$ | $D_3$ | $D_1$ | $D_3$ | $D_1$ | $D_3$ |
| 1. | *E. danricus* | 4.5 - 5.0 | 91.00 ± 6.60 | 65.00 ± 11.30 | 65.00 ± 7.70 | 50.00 ± 6.66 | 76.00 ± 5.92 | 56.00 ± 6.00 | 52.00 ± 4.39 | 39.00 ± 3.89 |
| 2. | *C. bacaila* | 5.0 - 6.5 | 67.00 ± 5.34 | 40.00 ± 9.20 | 52.00 ± 8.20 | 29.00 ± 3.36 | 45.00 ± 10.05 | 29.00 ± 6.50 | 30.00 ± 2.59 | 14.00 ± 5.18 |
| 3. | *C. fasciatus* | 4.0 - 6.0 | 93.00 ± 7.99 | 75.00 ± 7.96 | 53.00 ± 3.70 | 38.00 ± 6.26 | 57.00 ± 3.96 | 45.00 ± 5.40 | 40.00 ± 1.92 | 30.00 ± 5.20 |
| 4. | *Danio* sp. | 2.5 - 3.5 | 95.00 ± 3.27 | 70.00 ± 5.50 | 55:00 ± 10.35 | 26.00 ± 6.26 | 71.00 ± 6.35 | 57.00 ± 7.31 | 42.00 ± 4.70 | 30.0 ± 3.39 |
| 5. | *P. stigma* | 5.0 - 6.0 | 64.00 ± 6.30 | 43.00 ± 9.29 | 39.00 ± 5.45 | 15.00 ± 2.07 | 47.00 ± 10.37 | 30.00 ± 4.30 | 25.00 ± 7.96 | 14.00 ± 3.39 |
| 6. | *R. daniconius* | 4.5 - 5.5 | 61.00 ± 6.90 | 40.00 ± 6.36 | 32.00 ± 2.94 | 18.00 ± 3.30 | 46.00 ± 7.90 | 33.00 ± 4.08 | 24.00 ± 3.50 | 7.00 ± 2.97 |

**Table 3. Instar-wise consumption* of mosquito larvae by different indigenous larvivorous fishes**

| S. No. | Fish species | Larval instar | Active feeding period (Sep 1990) | | | | | | Lean feeding period (Jan 1991) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Without food | | | With food | | | Without food | | | With food | | |
| | | | $D_1$ | $D_2$ | $D_3$ | $D_1$ | $D_2$ | $D_3$ | $D_1$ | $D_2$ | $D_3$ | $D_1$ | $D_2$ | $D_3$ |
| 1. | *P. stigma* | I | 7.4 | 4.6 | 4.0 | 4.2 | 2.4 | 1.6 | 6.25 | 5.0 | 4.0 | 1.2 | 1.4 | 1.6 |
| | | II | 11.0 | 11.8 | 11.8 | 7.2 | 5.0 | 2.6 | 10.4 | 7.6 | 8.4 | 6.4 | 4.0 | 2.4 |
| | | III | 20.0 | 19.0 | 12.8 | 10.8 | 8.4 | 3.8 | 12.6 | 12.8 | 6.0 | 6.8 | 3.8 | 2.4 |
| | | IV | 25.8 | 20.8 | 14.8 | 17.0 | 13.2 | 7.4 | 18.0 | 13.6 | 11.0 | 11.0 | 8.6 | 7.0 |
| | | Total | 64.2 | 56.2 | 43.4 | 39.2 | 29.0 | 15.4 | 47.25 | 39.0 | 29.8 | 25.4 | 17.8 | 13.4 |
| 2. | *E. danricus* | I | 23.4 | 16.8 | 16.6 | 10.2 | 8.6 | 11.8 | 22.2 | 16.4 | 9.4 | 11.0 | 7.4 | 5.0 |
| | | II | 20.4 | 18.8 | 15.0 | 15.8 | 9.4 | 11.4 | 16.2 | 15.2 | 13.4 | 11.6 | 9.8 | 7.2 |
| | | III | 18.6 | 16.2 | 17.6 | 12.4 | 8.4 | 14.0 | 19.0 | 15.4 | 10.6 | 22.4 | 8.0 | 12.8 |
| | | IV | 28.4 | 17.6 | 15.8 | 26.2 | 12.2 | 12.4 | 18.6 | 21.2 | 22.6 | 18.6 | 15.2 | 14.2 |
| | | Total | 90.8 | 69.4 | 65.0 | 64.6 | 38.6 | 49.6 | 76.0 | 68.2 | 56.0 | 52.4 | 40.4 | 39.2 |
| 3. | *R. daniconius* | I | 13.2 | 5.8 | 11.2 | 7.0 | 5.6 | 5.0 | 12.2 | 3.4 | 6.0 | 5.2 | 2.6 | 1.8 |
| | | II | 13.2 | 8.6 | 6.8 | 5.4 | 6.8 | 2.8 | 14.0 | 6.0 | 10.0 | 6.4 | 3.4 | 1.0 |
| | | III | 17.0 | 16.8 | 13.2 | 8.4 | 6.0 | 4.6 | 10.0 | 11.4 | 8.6 | 5.6 | 7.0 | 2.4 |
| | | IV | 19.4 | 21.0 | 8.8 | 11.4 | 12.8 | 5.8 | 9.4 | 18.8 | 8.2 | 7.2 | 7.8 | 2.2 |
| | | Total | 62.8 | 52.2 | 40.0 | 32.2 | 31.2 | 18.2 | 45.6 | 39.6 | 32.8 | 24.4 | 20.8 | 7.4 |
| 4. | *C. fasciatus* | I | 23.2 | 14.8 | 19.8 | 12.8 | 12.8 | 5.0 | 12.0 | 7.2 | 10.2 | 10.6 | 9.6 | 6.2 |
| | | II | 19.8 | 13.8 | 16.8 | 12.6 | 6.0 | 5.2 | 11.4 | 9.4 | 12.2 | 9.6 | 4.4 | 7.4 |
| | | III | 28.2 | 17.8 | 17.0 | 13.8 | 14.8 | 15.2 | 15.0 | 10.8 | 10.8 | 10.4 | 13.0 | 8.2 |
| | | IV | 21.4 | 19.0 | 21.0 | 13.6 | 10.4 | 12.4 | 18.8 | 14.6 | 11.8 | 9.2 | 11.4 | 8.6 |
| | | Total | 92.6 | 65.0 | 74.6 | 52.8 | 44.0 | 37.8 | 57.2 | 42.0 | 45.0 | 39.8 | 38.4 | 30.4 |
| 5. | *Danio* sp. | I | 22.8 | 21.6 | 15.8 | 12.2 | 8.4 | 5.0 | 17.0 | 15.0 | 14.2 | 8.8 | 10.8 | 4.2 |
| | | II | 26.2 | 17.0 | 16.8 | 14.0 | 7.8 | 8.0 | 17.8 | 16.0 | 14.4 | 11.8 | 9.6 | 7.6 |
| | | III | 21.2 | 16.6 | 15.2 | 13.6 | 9.0 | 5.6 | 15.2 | 16.4 | 10.8 | 9.2 | 8.6 | 8.4 |
| | | IV | 24.6 | 16.2 | 22.0 | 15.0 | 9.4 | 7.6 | 18.6 | 16.4 | 17.6 | 12.6 | 11.4 | 9.8 |
| | | Total | 94.8 | 71.4 | 69.8 | 54.8 | 34.6 | 26.2 | 68.6 | 63.8 | 57.0 | 42.4 | 40.4 | 30.0 |
| 6. | *C. bacaila* | I | 17.2 | 12.2 | 8.6 | 13.8 | 6.2 | 3.4 | 10.6 | 7.6 | 6.4 | 7.0 | 4.4 | 1.8 |
| | | II | 15.8 | 11.8 | 10.2 | 10.2 | 8.4 | 6.6 | 6.2 | 9.2 | 8.4 | 6.8 | 8.8 | 2.2 |
| | | III | 17.4 | 16.0 | 11.2 | 15.0 | 10.6 | 8.2 | 13.4 | 9.6 | 6.8 | 8.2 | 4.4 | 5.2 |
| | | IV | 16.6 | 12.4 | 9.8 | 13.4 | 10.2 | 10.4 | 14.8 | 11.4 | 7.0 | 7.8 | 8.2 | 5.4 |
| | | Total | 67.0 | 52.4 | 39.8 | 52.4 | 35.4 | 28.6 | 45.0 | 37.8 | 28.6 | 29.8 | 25.8 | 14.6 |

* Average of five sets of experiments.

natural food. The difference in the feeding capacity of fishes in the months of September and January in the absence of natural food was highly significant ($P<0.001$).

## DISCUSSION

The survey of fishes in different aquatic habitats revealed a rich and diversified fish fauna in district Shahjahanpur. Most of the fish species were found in both lotic and lentic aquatic habitats. Fish species such as *E. danricus, C. fasciatus, A. mola, C. bacaila, Puntius* spp., *Channa* spp. and *E. daniconius* were abundant in all types of water bodies. This is because all these fish species are small (range only up to a few cm) and are not voracious or cannibalistic in their feeding habits, and therefore always sustain good population densities in an aquatic ecosystem as compared to voracious carnivorous fishes such as *W. attu, Mystus* spp. and *Channa* spp.

The larvivorous potential varied from one fish species to another because of different feeding habits and their mode of life. Allen[4] pointed out that the behaviour of the fish plays an important role in the selection mechanism but the behaviour of the food organism and the nature of the environment also have a role. Although 24 out of 35 fish species consumed mosquito larvae, yet on the basis of mosquito larval consumption, all these fish species cannot be recommended for mosquito control in view of the size of grown-up fishes and other factors such as feeding habits, breeding habits and their popularity among people as food, which becomes a limiting factor for their use in mosquito control. Except *E. danricus, C. bacaila* and *Danio* spp., all fish species are either column feeders or bottom feeders or are voracious carnivores and are highly destructive to small fishes of all species. Therefore, only six species were selected for detailed studies on their utility in mosquito control. Detailed investigations on the mosquito larval consumption of 6 selected larvivorous fish species showed that these fish species consumed all the larval stages of mosquito. However, they preferred III and IV instar larvae as mature larvae are more active or visible. The decline in larvivorous capacity in the presence of planktonic food might be because of selectivity/predilection for a particular food item available and accessible to fishes in the environment[5]. The decline in the feeding capacity of fishes during the lean feeding period may be due to low temperatures in January.

Nevertheless, no single fish species can meet the requirement of a wide variety of situations that exist in terms of mosquito breeding, but fishes could be selected for specific situations[6]. Therefore, suitable local larvivorous fishes can be categorised according to their utility in major mosquito breeding places. Fishes belonging to the genera such as *Esomus, Chela, Puntius, Danio* and *Rasbora* are useful in controlling mosquito breeding in freshwater bodies. Weed infestation may be problematic in some situations because of anaerobic decomposition. It is therefore suggested that the above-mentioned fish species should be introduced in freshwater bodies free from aquatic weeds. *Colisa* fish may be introduced in polluted waters such as drains and factory effluents, because these fishes have additional accessory respiratory organs

which enable them to obtain atmospheric oxygen for respiration during deoxygenation of the water. In the case of rivers and canals we have to rely on the natural fish fauna as introduction of larvivorous fishes is not feasible in such large aquatic habitats. But river-bed pools adjacent to the villages may be treated with locally available larvivorous fishes. However, considering their abundance, larvivorous potential, size and other biological factors the indigenous fishes such as *Colisa, Esomus, Danio, Chela, Puntius,* and *Rasbora* can be best utilised for controlling mosquito breeding in different aquatic habitats in an integrated vector control programme and thus the problem of insecticide resistance and environmental contamination can be avoided. In conclusion, the indigenous fishes have considerable larvivorous potential and so can play a significant role in mosquito control.

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# Malaria in Shankargarh PHC, Allahabad District (U.P.): A Clinical Report

R.N. YADAV*, S.N. TIWARI*, P.K. TYAGI†, A.K. KULSHRESTHA* and ANIL PRAKASH*

A longitudinal study of malaria incidence recorded at malaria clinic of MRC, Shankargarh, during 1988-1991 showed the immense popularity of the clinic in quarry area. In clinical cases, SPR and SfR gradually increased from 45.6 and 18.2 in 1988 to 52.5 and 34.5 respectively in 1991. Increase in malaria cases during the reporting period was mainly contributed by *P. falciparum* cases. Peaks of vivax and falciparum malaria were recorded in September and October respectively. Extended transmission in Shankargarh region might be attributed to the influx of quarry labourers after post-monsoon season. SfR for 0 to 1-year age group malaria cases was found to be relatively low as compared to the higher age groups. Only 56 per cent of malaria-positive patients reported in the clinic had fever.

**Key words:** Clinical malaria, *P. falciparum*, *P. vivax*

## INTRODUCTION

Shankargarh PHC of Allahabad district (U.P.) is known for the high incidence of malaria since the last decade. Therefore, the Malaria Research Centre, Delhi, opened a field station at Shankargarh and launched a science and technology project on mission mode, viz. integrated diseases vector control of malaria in April 1987. Under this programme, a malaria clinic was also opened at the field station in late 1987. The clinic started receiving a large number of fever cases for malaria confirmation. Fever cases included villagers of the whole PHC and migrant labourers working on stone quarries. A four-

* Malaria Research Centre (Field Station), Ram Bhawan, Shankargarh-212 108, India.
† Malaria Research Centre (Field Station), N-18, Civil Township, Rourkela-769 004, India.

year report of malaria incidence recorded at malaria clinic, MRC Shankargarh, is presented in this paper.

## MATERIALS AND METHODS

### Study area

The malaria clinic functioned from the building of Malaria Research Centre in Shankargarh. Shankargarh is a small town with a population of 25,000 and is situated 50 km southwest of Allahabad city. The town borders district Rewa (M.P.) towards southeast and district Banda towards southwest. PHC-Jasara is the adjoining PHC towards north of Shankargarh (Fig. 1). Villages in this region are scattered with poor communication. Population is mostly tribal, uneducated and backward with poor socio-economic status. There are numerous silica sand and hard stone quarries in this area and the characteristic feature of this area is the occurrence of large-scale labour movement from the adjoining districts/state every year for work in the quarries.

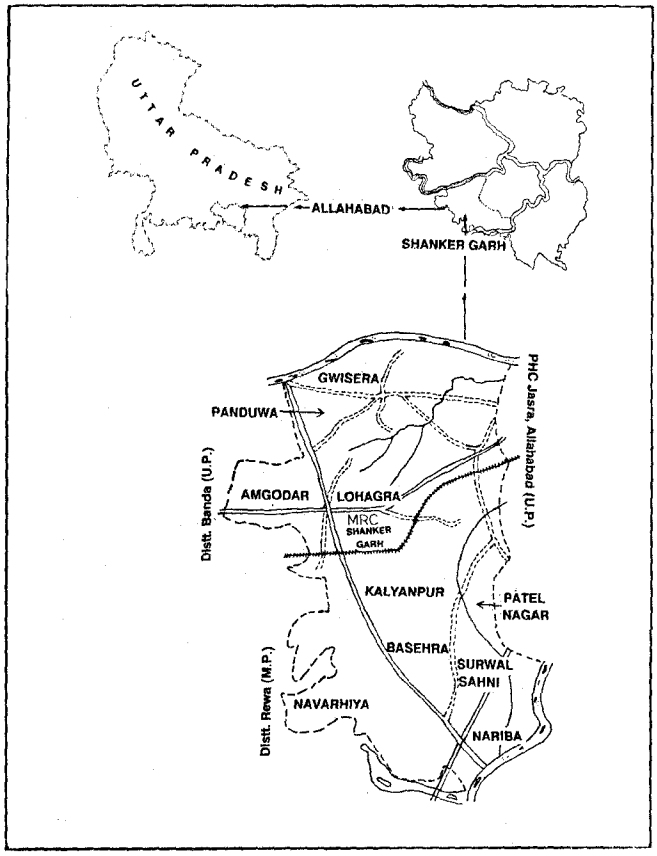


*Fig. 1:* Catchment areas of MRC malaria clinic in Shankargarh.

**Epidemiological investigations**

Initially all patients were registered and basic information such as name, father's name, name of the village, age, sex, history of fever and any other complaint of illness was recorded. Temperature was also recorded from some of the patients. Thick and thin blood smears were prepared by finger prick, and thin smears were fixed in methanol. Blood smears were stained with JSB and examined under oil immersion for malaria parasites. The positivity was confirmed by examining 50 fields of thick and 100 fields of thin blood smears.

All positive cases were treated the same day. An adult dosage of 1200 mg chloroquine in two equal, divided doses on two consecutive days was given to all malaria cases. In addition, *Plasmodium falciaprum* cases received 300 mg chloroquine on Day 2 and a single dose of 45 mg primaquine on Day 0. Likewise, *Plasmodium vivax* cases were given 75 mg primaquine in five equal, divided doses for five days. Malaria cases with mixed infection were treated for falciparum malaria.

RESULTS

During 1988 a total number of 7309 patients were examined. Out of these, 3334 cases were found positive for malaria parasite, i.e. 2001 for *P. vivax*, 1329 for *P. falciparum* and 4 for mixed infection. The slide positivity rate (SPR) and slide falciparum rate (SfR) were 45.6 and 18.2 respectively. However, in 1989 fever cases reported to the clinic were 8200 and of these, 4543 were malaria-positive. There were 1873 cases of *P. vivax,* 2663 of *P. falciparum* and 7 of mixed infection. The SPR and SfR recorded were 55.4 and 32.7 respectively. Likewise, during 1990, out of 12,209 patients reported to the clinic, 7019 were positive for malaria parasite. There were 2269 patients of *P. vivax* and 4748 were of *P. falciparum*. Two patients showed infection both for *P. vivax* and *P. falciparum*. The SPR and SfR were 57.5 and 38.9 respectively. In 1991, out of 15,473 blood smears examined, 8126 were found positive (SPR 52.5). Among positive cases, 2788 were *P. vivax,* 5314 were *P. falciparum* (SfR 34.5) and 24 cass were of mixed infection (Table 1).

Monthwise epidemiological data of the

**Table 1. Yearwise cases detected at malaria clinic, Shankargarh (1988 to 1991)**

| Year | BSC/E | +ve | Positive for | | | SPR | SfR | Pf% |
|---|---|---|---|---|---|---|---|---|
| | | | *Pv* | *Pf* | *Pv+Pf* | | | |
| 1988 | 7309 | 3334 | 2001 | 1329 | 4 | 45.6 | 18.2 | 39.9 |
| 1989 | 8200 | 4543 | 1873 | 2663 | 7 | 55.4 | 32.7 | 58.8 |
| 1990 | 12209 | 7019 | 2269 | 4748 | 2 | 57.5 | 38.9 | 67.7 |
| 1991 | 15473 | 8126 | 2788 | 5314 | 24 | 52.5 | 34.5 | 65.7 |
| Total | 43191 | 23022 | 8931 | 14054 | 37 | 53.3 | 32.6 | 61.2 |

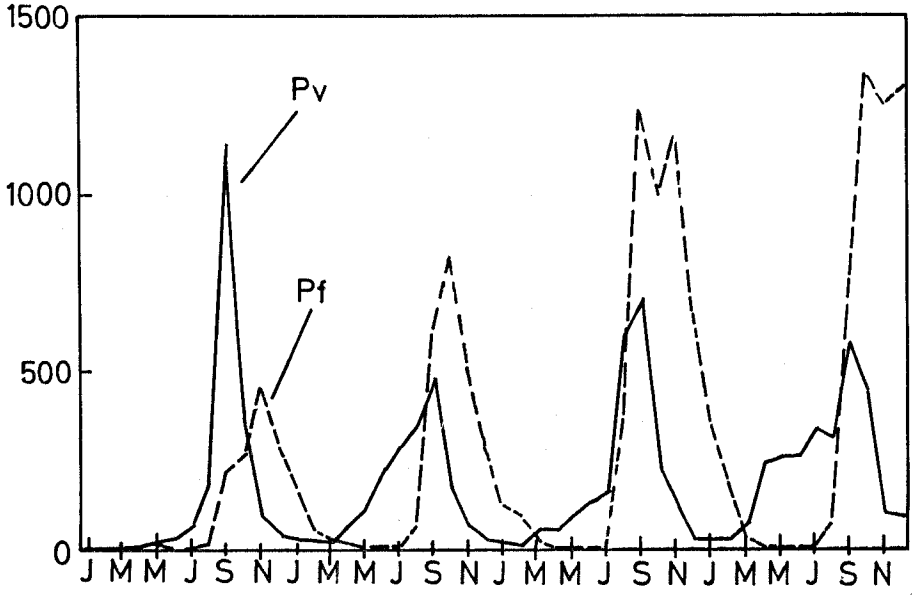


*Fig. 2:* Monthwise cases detected at malaria clinic, Shankargarh (1988 to 1991).

malaria clinic from 1988 to 1991 are shown in Fig. 2. It is evident that the positive cases detected in the clinic increased gradually every year. While there was only marginal increase in *P. vivax* cases (2001 in 1988 to 2788 in 1991), *P. falciparum* cases increased by over 4-fold (from 1329 in 1988 to 5314 in 1991). The pattern of malaria cases indicated that *P. falciparum* cases started building up from August onwards with a maximum in October/November, coinciding with a peak transmission period. In 1990, however, the maximum *P. falciparum* cases were registered in September. The highest SfR, however, was recorded usually in December, which was 51.4, 62.8, 69.2 and 66.4 for 1988, 1989, 1990 and 1991 respectively. Similarly, *P. vivax* cases also occurred in all the months of the year. *P. vivax* cases started increasing from April onwards and attained a peak always in September. Thereafter, *P. vivax* cases declined.

*P. falciaprum* cases monthwise and agewise with gametocyte rate are given in Table 2. The table shows more *P. falciparum* cases with respect to older persons. Thus, the slide falciparum rate for 0-1 year infants was comparatively lower (18.0) than for higher age groups. Out of the total 14,091 *P. falciparum* cases detected during 1988-1991 in the malaria clinic, 83, 664, 1610, 2581 and 9133 cases were recorded in patients of 0-1, 1-4, 4-8, 8-14 and ≥14 age groups respectively (Table 3). In 0-1 age group not a single *P. falciparum* case was recorded in April, May and June whereas in older age groups *P. falciparum* cases were recorded, though fewer, in these months also. Further, the proportions of *P. falciparum* cases recorded

**Table 2. Age and monthwise *P. falciparum* cases with gametocyte rate at malaria clinic, Shankargarh (1988 to 1991)**

| Month | 0-1 yrs | | 1-4 yrs | | 4-8 yrs | | 8-14 yrs | | ≥14 yrs | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pfr | Pfrg Pfg | Pfr | Pfrg Pfg | Pfr | Pfrg Pfg | Pfr | Pfrg Pfg | Pfr | Pfrg Pfg | Pfr | Pfrg Pfg |
| Jan | — | 1 | 13 | 3 | 44 | 14 | 76 | 26 | 364 | 126 | 497 | 170 |
| Feb | 1 | 1 | 14 | 10 | 17 | 11 | 50 | 22 | 197 | 72 | 279 | 116 |
| Mar | 1 | 1 | 5 | 3 | 8 | 10 | 7 | 10 | 54 | 31 | 75 | 55 |
| Apr | — | — | 1 | 1 | 2 | 2 | 5 | 3 | 22 | 21 | 30 | 27 |
| May | — | — | — | 1 | 1 | 2 | 2 | 5 | 11 | 11 | 14 | 19 |
| Jun | — | — | — | — | — | 3 | — | 1 | 11 | 10 | 11 | 14 |
| Jul | 1 | — | — | 1 | 3 | 2 | 1 | — | 14 | 10 | 19 | 13 |
| Aug | 1 | — | 25 | 3 | 57 | 8 | 67 | 24 | 296 | 68 | 446 | 103 |
| Sep | 17 | 1 | 135 | 11 | 276 | 53 | 399 | 70 | 1543 | 282 | 2370 | 417 |
| Oct | 16 | 3 | 140 | 45 | 318 | 74 | 528 | 127 | 1797 | 406 | 2799 | 655 |
| Nov | 20 | 4 | 106 | 38 | 328 | 82 | 479 | 140 | 1774 | 437 | 2707 | 701 |
| Dec | 13 | 2 | 103 | 26 | 247 | 48 | 419 | 120 | 1266 | 310 | 2048 | 506 |
| Total | 70 | 13 | 542 | 142 | 1301 | 309 | 2033 | 548 | 7349 | 1784 | 11295 | 2796 |
| Gametocyte rate | 15.7 | | 20.8 | | 19.2 | | 21.2 | | 19.5 | | 24.8 | |

**Table 3. Age and sexwise malaria cases detected at malaria clinic, Shankargarh (1988 to 1991)**

| Age group (yrs) | Sex | B.S. collection | Positive | | | | SPR | SfR |
|---|---|---|---|---|---|---|---|---|
| | | | *Pv* | *Pf* | Mix | Total | | |
| 0-1 | Male | 332 | 91 | 37 | — | 128 | 44.6 | 17.2 |
| | Female | 241 | 74 | 44 | 2 | 120 | 49.8 | 19.1 |
| 1-4 | Male | 1647 | 421 | 408 | 1 | 830 | 50.4 | 24.8 |
| | Female | 1089 | 276 | 273 | 2 | 551 | 50.6 | 25.3 |
| 4-8 | Male | 2984 | 735 | 959 | 1 | 1695 | 56.8 | 32.2 |
| | Female | 2064 | 528 | 643 | 7 | 1178 | 57.9 | 31.5 |
| 8-14 | Male | 5024 | 1157 | 1725 | 8 | 2890 | 57.5 | 34.5 |
| | Female | 2537 | 607 | 846 | 2 | 1455 | 57.4 | 33.4 |
| ≥14 | Male | 19021 | 3462 | 6393 | 11 | 9866 | 51.8 | 33.6 |
| | Female | 8252 | 1580 | 2726 | 3 | 4309 | 52.2 | 33.1 |

with gametocytes was relatively lower, i.e. 15.7% in patients of 0-1 year age groups. In other age groups, i.e. 1-4, 4-8, 8-14 and above, the proportions of *P. falciparum* cases with gametocytes recorded were 20.8, 19.2, 21.2 and 19.5% respectively. There was no significant difference in SPR and SfR for males and females in patients of any age group (Table 3).

Out of 4843 malaria cases whose clinical features were analysed, 3353 (69.2%) had headache, 601 (12.4%) cough, 153 (3.2%) diarrhoea and 736 (15.2%) vomitting. In regard to the clinical feature of headache more than 90% *P. falciparum* patients had headache, whereas its extent was relatively less (58%) in *P. vivax* patients (Table 4). In respect of other symptoms like cough, diarrhoea and vomitting, patients of both *P. vivax* and *P. falciparum* suffered equally. Out of 2400 malaria cases whose temperature was recorded, 476 (19.8%) recorded temperatures between 98 and 100°F, 384 (16%) between 102 and 104°F, 476 (19.8%) between 100 and 102°F, and 7 (0.3%) between 104 and 106°F. The remaining 1057 cases (44%) were without noticeable fever at the time of examination. More number of *P. falciparum* patients were noticed without fever (21%) than those of *P. vivax* patients (9.5%) when they reported at the clinic (Table 4).

## DISCUSSION

A majority of the patients reported at the malaria clinic were from Shankargarh township and its rural areas within a radius of 20-30 km. Shankargarh block is characterized by numerous quarries which provide an ideal breeding ground for anopheline mosquitoes, particularly in monsoon and post-monsoon season. Moreover, owing to the quarring activities, there is a continuous labour movement in this region from the adjoining areas serving as an additional parasitic reservoir. Malaria cases detected in the clinic gradually increased from 3334 in 1988 to 8126 in 1991, resulting in a considerable rise in SPR and SfR (45.6 and 18.2 in 1988, 52.5 and 34.5 in 1991). There appears to be a direct correlation between increase in malaria cases and the number of labourers coming for quarrying over the years. Regular surveys of labourers working in stone quarries revealed that labour population increased gradually from 20,101 in 1988 to 32,827 in 1990. Since bulk of the migratory labourers usually comes during post-monsoon season (September-October) and returns to their na-

**Table 4. Clinical features of malaria patients reported in malaria clinic, Shankargarh (in per cent)**

| Species | Temperature (°F) | | | | | Headache | Cough | Diarrhoea | Vomitting |
|---|---|---|---|---|---|---|---|---|---|
| | 96 to 98 | 98 to 100 | 100 to 102 | 102 to 104 | 104 to 106 | | | | |
| *P. vivax* | 9.5 | 31.8 | 32.1 | 26.3 | 0.3 | 58.2 | 14.1 | 3.9 | 14.3 |
| *P. falciparum* | 20.8 | 31.5 | 25.6 | 21.7 | 0.3 | 91.1 | 17.7 | 3.9 | 18.3 |

tive places in March-April there is a prolonged transmission which appears to be one of the main reasons for gradual rise in malaria in this region. However, other factors such as the popularity of the malaria clinic, which provides prompt and correct diagnosis of malaria and free of cost treatment, the phenomenon of drug resistance, and other factors might also be responsible for increase in malaria cases recorded at the clinic. Sharma *et al.*[1], Choudhary *et al.*[2] and Malhotra *et al.*[3] have reported a similar increase in malaria cases from Bhabar and Terai areas of U.P., which was, however, due to the cyclical epidemic.

Another striking feature is that the increase in malaria cases is due mainly to *P. falciparum*. From 1988 to 1991 *P. falciparum* cases increased by 4-fold as compared to nearly ½-fold increase in *P. vivax* cases. The percentage of *Pf* cases increased gradually from 39.9 in 1988 to 58.8 in 1989, 67.7 in 1990 and 65.7 in 1991. The presence of drug-resistant *Pf* strains and its gradual spread might be contributing to the dissemination of *P. falciparum* cases. *In-vivo* and *in-vitro* resistance study carried out in 1988 reveald 33% resistance in Shankargarh block[4]. It is suspected that the labourers coming from district Mirzapur (U.P.) and district Rewa (M.P.), which are the known *P. falciparum* resistant areas[5], might be responsible for spreading the resistant strains of *P. falciparum* in this area. Similarly, introduction of nonimmune/relatively less immune labourers might be increasing the susceptibility of the population, thereby, promoting the severity of transmission in this area. The present observation of a gradual increase in *Pf* malaria cases in Shankargarh area is in conformity with the observations of Sharma *et al.*[1,6], Choudhary *et al.*[2] and Malhotra *et al.*[3], who reported that the falciparum malaria is increasing rapidly during the last few years. As far as *P. vivax* cases are concerned they are still susceptible to chloroquine in this area. During pre-monsoon dry months *P. vivax* malaria is mainly contributed by the relapse cases. However, in monsoon/post-monsoon season, transmission adds to fresh cases.

In areas with prolonged transmission the longer duration of gametocytaemia in untreated persons and lesser stimulation of immune response have been reported to strengthen *P. falciparum*. Further, the transmission rate of malaria within the community produces a collective immune response which at a high level decreases the severity of the infections of older age groups while the burden of disease falls on infants and children. The parasite positivity in infants represents the transmission rate whereas the proportion of acute cases and gametocyte rate are the indicators of level of endemicity[7]. In the clinic, *P. falciparum* cases appeared almost throughout the year in all age groups, except 0-1 year age group. However, in infants, *P. falciparum* malaria cases were observed mostly during September, October, November and December, indicating the transmission season in this area. Similarly, quite a high gametocyte rate observed for infants (15.7) suggests a high degree of endemicity in this region.

Malhotra *et al.*[3] reported that children up to 1-year age group are less prone to malaria than higher age groups. Further, he observed

no difference in the incidence of malaria in males and females. Our study also substantiates these findings.

Headache, bodyache, fever of varying duration and intensity, shivering, loss of appetite, cough, etc. are the clinical symptoms which have been observed among malaria cases at MRC clinic in Delhi[8]. In our investigations, 56% of the malaria pateints showed fever and the most prominent temperature range recorded was 98-102°F. Other symptoms observed were cough, headache and diarrhoea. Vomitting was also noticed in 15.2% cases which might be the consequence of gastric irritation due to malaria infection. Patients of both *P. vivax* and *P. falciparum* presented more or less similar clinical features except that a larger proportion of *P. falciparum* patients (91%) complained of headache as compared to only 58% *P. vivax* patients. Another difference observed in clinical symptoms of *Pv* and *Pf* patients was that the patients with unnoticeable fever (96 to 98°F) were more in the case of *Pf* infection (21%) than in *Pv* (9.5%), thereby reflecting the presence of a higher number of asymptomatic cases in the case of *P. falciparum* malaria in this region.

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# Application of Bactoculicide (*Bacillus thuringiensis* H-14) for Controlling Mosquito Breeding in Industrial Scrap at BHEL, Hardwar (U.P.)

VIRENDRA K. DUA*, S.K. SHARMA* and V.P. SHARMA†

Bactoculicide (*Bacillus thuringiensis*) was evaluated in field trials for controlling mosquito breeding of *Aedes*, *Culex* and *Anopheles* in industrial scraps such as broken heavy machine parts, iron moulds and discarded drums. A dose of 0.5 g/m$^2$ was controlled 96-100% mosquito breeding up to five weeks.

**Key words:** *Aedes*, *Anopheles*, *Bacillus thuringiensis*, Biological control, *Culex*

## INTRODUCTION

Owing to the growing problem of widespread insecticide resistance in vectors, high refusal rate for indoor sprays, soaring prices of chemical insecticides and the behaviour of the mosquitoes, the use of adulticides has become less effective and uneconomical. Therefore, bioenvironmental control of malaria model based on non-insecticidal approach was developed in Kheda district of Gujarat[1] and later this strategy was extended to Bharat Heavy Electricals Ltd. (BHEL), an industrial complex at Hardwar[2]. One of the major breeding sites in BHEL campus was a large number of industrial scraps such as broken heavy machine parts, iron moulds and discarded drums (Fig. 1), where *Aedes* and *Culex* were breeding mainly along with a small percentage of *Anopheles* mosquitoes because of rain-water collection in these containers. Attempts to control mosquito breed-

* Malaria Research Centre (Field Station), BHEL Complex, Ranipur, Hardwar-249 403, India.
† Malaria Research Centre, 20, Madhuvan, Delhi - 110 092, India.

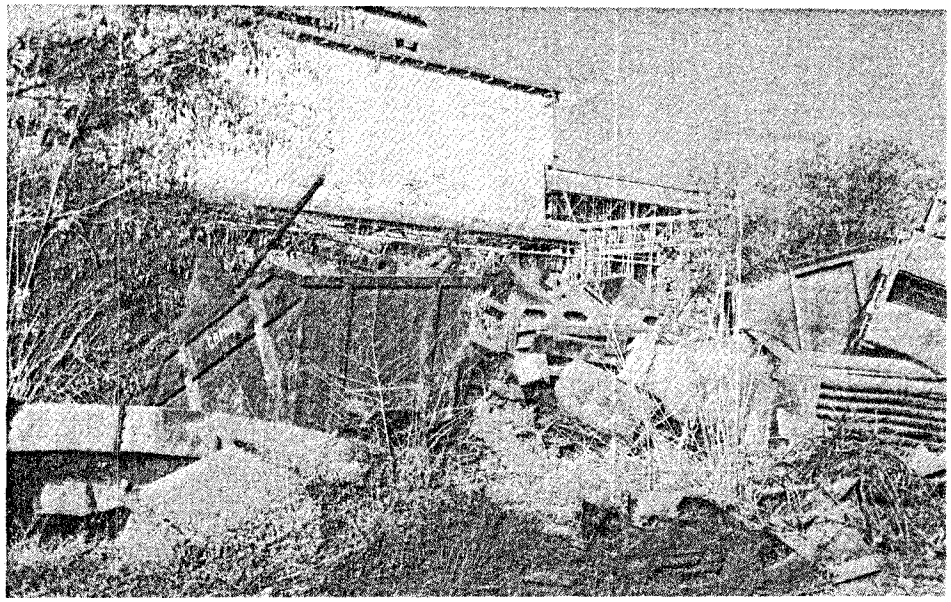

*Fig. 1:* Industrial scrap at BHEL, Hardwar.

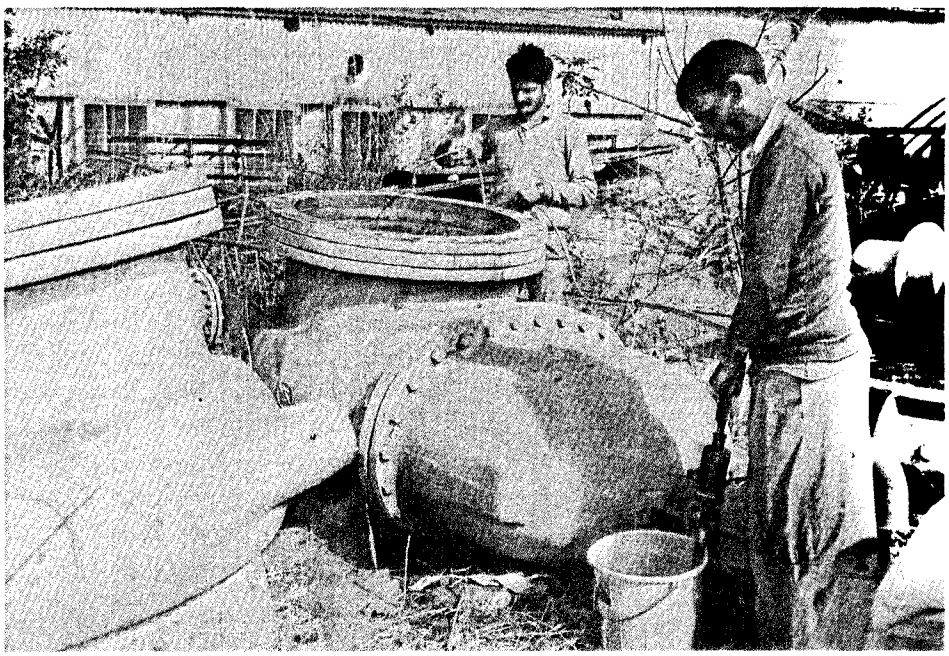

*Fig. 2:* Application of Bactoculicide in industrial scraps.

ing in these habitats by mechanical methods or by application of expanded polystyrene (EPS) beads[3] and larvivorous fishes were unsuccessful. Therefore, Bactoculicide (*Bacillus thuringiensis*) was evaluated for its efficacy to control mosquito breeding in factory scraps in the industrial area. The results of the study are presented in this paper.

## MATERIALS AND METHODS

Bactoculicide is a formulation of *Bacillus thuringiensis* var. *israeliensis* H-14, a product manufactured by Ministry of Chemical Industry, Bordsk Chemical Plant, Moscow, Russia, and was supplied by M/s. Chemical International Ltd., New Delhi. The experiment began in the fourth week of August, 1991 and 73 scrap containers found positive for mosquito breeding were selected for the treatment of Bactoculicide, and 10 containers were kept as control for comparison as well as for recording natural fluctuations in population during the study period. Pretreatment (D0) larval density (I+II and III+IV instars and pupae) of *Anopheles*, *Aedes* and *Culex* was measured by using a 10 cm diam dipper of 250 ml capacity and an average of 5 dips/container was taken for calculation of mean larval density of experiental and control habitats. Larval samples were brought to the laboratory for mosquito emergence and subsequent identification. Suspension of Bactoculicide (0.5%) was prepared by mixing 5 g powder formulation in one litre of water and stirring it till a uniform suspension was obtained. The suspension was sprayed over the water surface of the experimental containers (Fig. 2) with the help of a stir-up pump at the dose of 0.5 g/m$^2$ surface area. The post-treatment larval density was recorded at 24-h intervals for the first 3 days and then monitored on Days 7, 10, 14, 18 and 24 and thereafter at weekly intervals till the impact persisted. All observations were recorded in the pro forma and per cent reduction in III and IV instar larvae was calculated using the formula described by Mulla[4].

## RESULTS AND DISCUSSION

A breeding survey of 1701 scrap containers inside the factory area of BHEL, Hardwar, during 1991 revealed 120 (7%) positive for mosquito larvae. Out of which, 66 (55%) were positive for *Aedes albopictus* and *Ae. aegypti*, 50 (41%) for *Culex quinquefasciatus* and 4 (3.3%) together for *Anopheles subpictus*, *An. culicifacies* and *An. stephensi*. The results of application of Bactoculicide in experimental scrap containers along with their comparison with control habitats are given in Table 1. The larval density (III+IV instar) became zero within 24 h after the application of Bactoculicide in all experimental habitats and remained nil until Day 10. Moreover, between 96.6 and 100% reduction in larval density was recorded up to 5th week in all the experimental habitats as compared to control ones. After 5th week, the effect of Bactoculicide decreased as is evident by 90% reduction in larval density in 6th week.

Industrial scraps are potential breeding sources of *Aedes* and *Culex* mosquitoes and create mosquito nuisance in the factory area and indirectly affect the efficiency of workers. Mechanical methods of control like inverting and emptying required a great deal of efforts

**Table 1. Field evaluation of Bactoculicide (*Bacillus thuringiensis* var. *israeliensis* H-14) against mosquito larvae* in industrial scraps**

(Dosage : 0.5 g/m$^2$; Observation period : 28.8.91 to 9.10.91)

| Day/week | Mean larval density per dip (III + IV instar only) | | |
|---|---|---|---|
| | Control | Experimental | |
| D0† | 1.5 | 6.0 | |
| D1‡ | 1.0 | 0 | (100) |
| D2 | 1.0 | 0 | (100) |
| D3 | 3.0 | 0 | (100) |
| D7 | 3.0 | 0 | (100) |
| D10 | 4.5 | 0 | (100) |
| D14 | 6.5 | 0.6 | (97.7) |
| D18 | 6.0 | 0.8 | (96.6) |
| D24 | 2.5 | 0.16 | (98.4) |
| 5th week | 2.0 | 0 | (100) |
| 6th week | 1.5 | 0.6 | (90) |

**Aedes, Culex* and scanty *Anopheles*; †Pre-treatment; ‡Post-treatment.

Figures in parentheses indicate per cent reduction of III + IV instar larvae based on untreated control calculated by Mulla's formula[4].

as well as the attention of factory workers which proved unsuccessful. Larvivorous fishes did not survive in these habitats and application of expanded polystyrene beads had very little impact. Thus, Bactoculicide appeared to be the best solution for controlling mosquito breeding in such problematic habitats. *Bacillus thuringiensis* is a known potent microbial insecticide against mosquito larvae[5] and also is very safe for other non-target organisms, including man[6]. The formulations of this bio-cide, which are very stable even under tropical storage conditions, have been evaluated against a number of immatures of different mosquito species under laboratory and field conditions with varying results[7-10]. A very important aspect of biocide application is that the susceptibility of mosquito larvae to these microbial agents does not seem to be affected by the resistance of these larvae to conventional insecticides[11].

Therefore, use of Bactoculicide can be of great help in public health programmes. In our investigation, Bactoculicide (*B. thuringiensis*) was found to control mosquito breeding in containers (industrial scraps) successfully. Although this biocide does not recycle in natural habitats, yet one application was found effective to control mosquito breeding up to five weeks.

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# Role of Macrophages in Experimental Malaria: I. Development of Immunobioassay Indicators

C.R. PILLAI* and N.N. SINGH†

The role of macrophages in immunogenic mechanisms of malaria was studied. The first part of the study aimed at development of indicators for assessing immunobioassay. Accordingly, data on the natural course of lethal *Plasmodium berghei* infection in mice were collected, and baseline estimates of a set of indicators were made. The indicators along with their estimated means are: prepatent period (PP), 2.57±0.06 days; survival period (SP), 17.63±0.29 days; median survival day (MSD), 17.20 days; and parasite density 24 h before death (*K*), 3582.6 infected RBC/$10^4$RBCs. The probable role of immunogenic mechanisms judged indirectly by course of parasitaemia in different phases is discussed.

**Key words:** Immunobioassay, Macrophages, Malaria

## INTRODUCTION

Because of the development of drug resistance and resurgence of malaria, there is a need to develop immunological methods, such as vaccination, for management of malaria in the community. To develop a potential vaccine there is a need to carry out comprehensive research on immunity of malaria. For such a research, a set of indicators is essential. An attempt, therefore, has been made to study systematically the course of induced *P. berghei* infection in mice with a view to developing indicators for immunobioassay.

## MATERIALS AND METHODS

Eighty-five male Swiss albino mice, 6-8 weeks old, with a mean weight 30.14±0.24 g

* Malaria Research Centre, 2, Nanak Enclave, Delhi-110 009, India.
† BA/13-A (Phase I), Ashok Vihar, Delhi-110 052, India.

were used. The mice were procured from the Disease Free Small Animal House, Haryana Agricultural University, Hissar. The *P. berghei* NK65 strain obtained from PGI, Chandigarh, was used to infect the mice by inoculating intraperitoneally, the dose being $1 \times 10^4$ parasitized erythrocytes. Parasitized erythrocytes were collected during the ascending phases of parasitaemia in anticoagulant and kept at 4°C till inoculation. The course of infection was monitored by microscopic examination of blood smears collected at every 24 h intervals till death of the animals. The smears were stained with JSB stain[1,2]; infected erythrocytes were counted and recorded per $10^4$ RBCs.

The data on parasite count were transformed in log scale to estimate the parameters. The mortality rate of the host (mice) was also estimated at 24 h intervals after infection. To get a close approximation of the survival period, 0.5 day was added to the last day on which the animal was found alive, e.g. an animal dying sometime between Day 19 and 20 was considered to survive for 19.5 days. Cumulative mortality rate over days was taken into account for estimating the median survival day (MSD) by the graphic method.

The course of parasitaemia was studied from a graph based on daily geometric mean of parasite count. From the characteristics of the curve a set of indicators, viz. prepatent period (PP), survival period (SP) and parasite density 24 h before death ($K$) were developed.

To estimate these indicators, the length of prepatent period, the level of daily parasitaemia and survival period were recorded for each animal. The data were used after appropriate transformation to estimate the parameters of central tendencies with their standard errors and 95% fiducial limits, and the coefficient of variation following standard statistical methods.

## RESULTS AND DISCUSSION

*Plasmodium berghei* was selected for the immunological study as it is highly lethal to the host (albino mice) and so the information derived could probably be of direct relevance to semi-lethal *P. falciparum* infection in man. Further, the parasite and the host are easy to maintain and manipulate in experiments, at low cost.

The inference based on experimental immunology needs estimation of the characteristic response and its comparison with that of the control group of animals or baseline indicators. The estimates of different indicators reported were based on 85 animals and could be used as baseline statistics.

The course of untreated *P. berghei* infection (Fig. 1) was almost similar to the findings of Ramakrishnan and Prakash[3]. In our study the course of infection showed four characteristic segments, namely lower asymptotic (phase I), steep growth (phase II), transient upper asymptotic (phase III) followed by a slow increase in parasite density, terminating at the death of the mice (phase IV). During the first phase only innate immunity of the host operated, and the parasite density remained below the level of microscopic detection. This phase was called prepatent period.

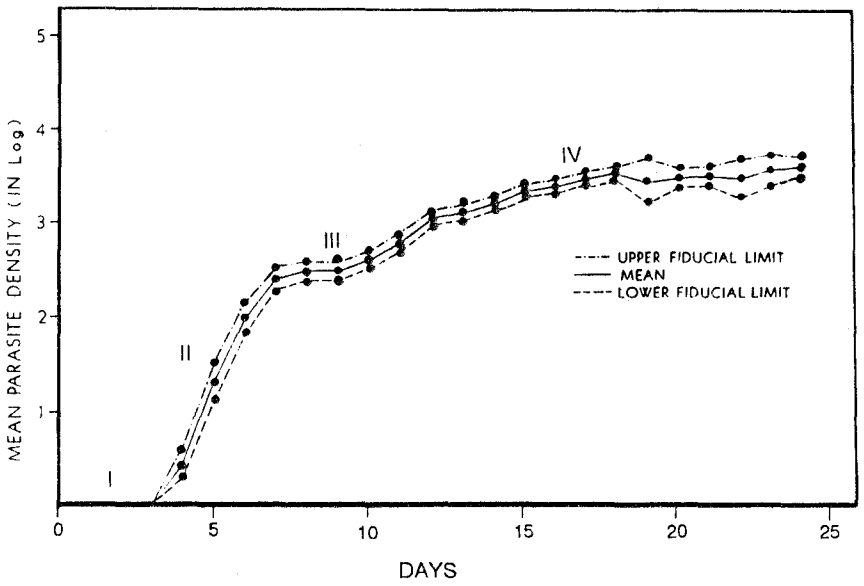


*Fig. 1:* Course of infection of *P. berghei* in untreated albino mice infected with $1 \times 10^4$ parasitised erythrocytes showing four characteristic phases.

The mean prepatent period (PP) was estimated as 2.59±0.06 days with upper and lower 95 per cent fiducial limits as 2.69 and 2.48 days. The median PP was 2.15 days. The observed range of prepatency was 2-4 days and the coefficient of variation (CV) was 19.92 per cent (Table 1). The duration of PP (3 days) could depend on the dose of *P. berghei* and innate immunity level of the host. Factors like weight, age etc. could also affect this period. Absence of this phase in the earlier study (Fig. 2) is due to a higher dose of inoculum.

The steep growth (phase II) was of 4 days' duration. The immunogenic mechanism of the host was activated in this phase which became operative subsequently in phase III. The steep growth of parasite population in phase II could be due to low level of specific immunity induced at phase I.

Phase III was a transient one of 2-3 days' duration and occurred after 7 days of inoculation. This phase was also observed from the data of Ramakrishnan *et al.*[4] (Fig. 2); it was also of 2-3 days' duration and had occurred after 7 days of inoculation (infection). In view of its consistency, it could be considered characteristic of *P. berghei* infection in mice. It was found to be independent of the dose of inoculum (Fig. 2). During this phase, equilibrium between the parasite and the host immunity was established. The equilibrium, however, later shifts in favour of parasites.

**Table 1. Baseline summary statistics on *P. berghei* infection in untreated mice**

| Indicator (unit) | n | Means with 95% fiducial limits | | | Median† | Range | CV |
|---|---|---|---|---|---|---|---|
| | | UL | Mean | LL | | | |
| Prepatent period (day) | 85 | 2.69 | 2.59±0.06 | 2.48 | 2.15 | 2-4 | 19.92 |
| Patency (day) | 85 | 15.13 | 14.56±0.29 | 13.99 | 13.75 | 9-21 | 18.35 |
| Survival period (day) | 85 | 18.19 | 17.63±0.29 | 17.06 | 17.20 | 12-24.5 | 15.14 |
| Parasitaemia per $10^4$ RBC 24 h before death (in log)* | 85 | 3828.25 (3.58) | 3582.61 (3.55±0.01) | 3352.74 (3.53) | 2691.53 (3.43) | 1499.68-6998.42 (3.18-3.85) | 3.81 |

*Figures in parentheses are in log units; †Estimated graphically; UL—Upper limits; LL—Lower limits; n—Number of animals; CV—Coefficient of variation.

In phase IV the growth rate of parasitic population was slower than that in phase II and continued till death of the host. The slow rate of growth could be due to the operation of induced immunity developed during earlier phases. The duration of phase IV was of

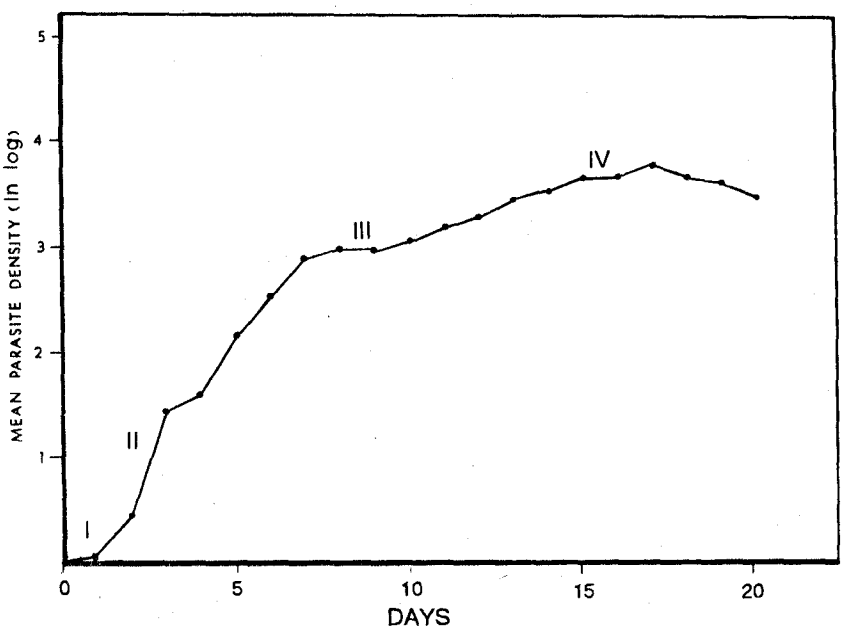


*Fig. 2:* Course of infection of *P. berghei* in untreated albino mice infected with $5 \times 10^6$ parasitised erythrocytes showing four characteristic phases (Based on the data of Ramakrishnan *et al.*[4]).

15 days commencing from Day 9 after inoculation. The mean patency period (phase II-IV) was 14.56±0.29 days with 15.13 and 13.99 as upper and lower 95 per cent fiducial limits. The median patency period was of 13.75 days. The patency period ranged from 9 to 21 days. The coefficient of variation (CV) was estimated as 18.35 per cent (Table 1).

The mean survival period (SP) (phases I-IV) was estimated as 17.63±0.29 days with upper and lower 95 per cent fiducial limits as 18.19 and 17.06 respectively. The observed range of the survival period was 12.5 - 24.5 days and the estimated CV was 15.14 per cent (Table 1). SP, being susceptible to extreme values of observation, was supplemented by MSD, which was 17.20 days.

The mean log parasitaemia 24 h before death *(K)* was estimated as 3.55±0.01 with 3.58 and 3.53 as upper and lower 95 per cent fiducial limits respectively. In actual units the mean parasite density was 3582.61 infected RBC/$10^4$RBCs with upper and lower 95 per cent fiducial limits as 3828.25 and 3352.74. The median *K* was 3.43, i.e. 2691.53 infected RBC. The range of *K* was 3.18 to 3.85, i.e. 1499.68 to 6998.42 infected RBC in actual units. The coefficient of variation in *K* was 3.81 per cent (Table 1).

Based on the course of infection the stable baseline immunobioassay indicators, viz. PP, SP, MSD and *K*, were estimated.

The survival period, being the period commencing from infection to the death of mice, contained all the four phases of the course of infection. Since *P. berghei* is a highly lethal infection in mice, this indicator is stable and sensitive enough for study of experimental immunity. The estimates of this indicator are, however, highly susceptible to extreme values of survival. Hence SP should be used as a complement of MSD, which is a more precise estimator.

*P. berghei* is a lethal infection of mice and hence the death of the mice would be directly or indirectly attributed to the density of infection. The lethal density of infection, 24 h or less, before death (mean *K*) could be taken as the mean lethal dose of parasitaemia. This indicator reflects the forces of innate biotic potential and mortality of the parasite in a given host. It also represents indirectly the force of immunity operating in the host.

The sensitivities of indicators were judged by the estimates of CV; the lower the value of CV the lesser the variability and consequently the greater the amount of information and sensitivity. The order of amount of information content in and sensitivity of the indicators were thus *K*>SP>PP.

The baseline estimates given in Table 1 are likely to be affected by genetic variability of the host and parasite and other factors.

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# *In-vivo* and *in-vitro* Sensitivity of *Plasmodium falciparum* to Chloroquine at Indian Oil Corporation, Mathura (U.P.)

VIRENDRA K. DUA*, PARITOSH K. KAR*, SURESH KUMAR* and V.P. SHARMA†

*In vivo* and *in vitro* susceptibility of *Plasmodium falciparum* to chloroquine were conducted at Indian Oil Corporation (IOC), Mathura, India. 18 out of 31 cases showed resistance [minimum inhibitory concentration (MIC) 8 pmol] in *in vitro* study. EC50 and EC90 values estimated from log-probit analysis for resistant isolates were 0.66 and 1.44 μM/litre, and for sensitive isolates 0.28 and 0.96 μM/litre blood respectively. *In vivo* tests identified 13 cases (40.62%) as resistant and 19 cases (59.73%) as sensitive out of 32 cases. All the cases belonged to IOC, Mathura complex, or its vicinity.

**Key words:** Chloroquine, Drug sensitivity, *Plasmodium falciparum*

## INTRODUCTION

Most of the industrial complexes are located in areas with a moderate to high malaria incidence due to construction work, and infiltration of the labourers or factory workers from different endemic areas of the country. All industrial complexes have well-equipped hospitals for diagnosis and treatment of malaria cases. However, most clinicians prescribe antimalarials regardless of the species of the parasite or sensitivity of *P. falciparum*. A focus of chloroquine re-sistance to *P. falciparum* has been reported in National Thermal Power Corporation (NTPC), Shaktinagar Complex[1], Mirzapur district, U.P. Recently, cases of *P. falciparum* resistant to chloroquine were found in Bharat Heavy Electricals Limited (BHEL) area, Hardwar[2], where most of the cases were im-

* Malaria Research Centre (Field Station), BHEL Complex, Ranipur, Hardwar - 249 403, India.
† Malaria Research Centre, 20, Madhuvan, Delhi - 110 092, India.

ported from the other endemic areas of the country. In continuation of our work on bioenvironmental control of malaria in different industrial complexes[3,4], it has been observed that the malaria incidence, particulary due to *P. falciparum*, at Indian Oil Corporation (IOC), Mathura complex, has been increasing rapidly during the last five years. Earlier it had been reported[5] that all *P. falciparum* cases from this area were sensitive to chloroquine. However, during the discussion with the Chief Medical Officer of the hospital, it was found that about 30% of the cases were not responding to chloroquine. Hence, it was necessary to test the sensitivity of *P. falciparum* to chloroquine both *in vivo* and *in vitro* in order to know the response of the parasite to the drug for proper treatment and for removing the resistant foci, if any, in the IOC area. The results are presented here.

## MATERIALS AND METHODS

### Study area

Mathura refinery is one of the five refineries of the Indian Oil Corporation Ltd., a major public sector oil-processing industry of India. The township is well planned with 2480 houses and a total population of about 16,000. It has a 25-bed hospital managed by 12 qualified doctors with subordinate staff. The hospital has its own pathology laboratory with two technicians. All clinically diagnosed symptomatic malaria fever cases are screened microscopically and only malaria-positive cases are given radical treatment as per standard norms. The study was conducted during the months of November - December 1990.

*In vivo* test (WHO 28-day extended test) for chloroquine sensitivity was started with 36 selected patients with a history of no reinfection during the study, after ascertaining that no chloroquine had been taken during illness by examination of urine for the chloroquine excretion[6]. Each patient received a total dose of 1500 mg (25 mg/kg body weight) of chloroquine base (600 D0, 600 D2 and 300 D3) followed by a single dose of 45 mg primaquine. Absorption of chloroquine was also confirmed by the urine test[6] on Day 3 (D3). 32 out of 36 patients were followed for 28 days. Blood smears were collected on D0, D2, D7, D14, D21 and D28 and whenever the patient complained of fever after the completion of prescribed doses. Asexual parasites were examined from Giemsa-stained smears. Chloroquine-resistant cases were treated with a single dose of sulfalene (1000 mg) plus pyrimethamine (50 mg) combination (Metakelfin) along with primaquine (45 mg). Parasite density was determined in all the cases on D0 and D2 for assessing the level of resistance by 7-day sentinel system.

Micro *in-vitro* tests for chloroquine were conducted with infected blood samples collected from 38 selected patients in predosed microculture plates supplied by WHO and the procedures for incubation and staining of pre- and post-incubation smears were the same as described earlier[7,8]. A test was considered valid when at least 10 per cent schizont maturation was observed in post-incubation control wells[9]. Schizont maturation at 8 pmol of chloroquine was considered an indication of resistance[10]. Minimum inhibitory concentrations (MICs) of the drug were assessed by microscopic examination of post-incuba-

tion smears. The results of *in-vitro* tests were analysed by probit analysis of log-dose response test[11].

## RESULTS

The malaria situation at IOC, Mathura, is given in Table 1, which shows that the incidence of the disease is static in the complex during last six years. During the investigation period (November-December 1990), of the 261 blood smears collected, 68 were positive for malaria parasites and 43 were of *P. falciparum.*

### *In-vitro* test

The results of *in-vitro* tests are given in Table 2. Out of 38 samples tested, schizont maturation in control wells occurred in 31 samples (81.57%). The MICs in the 13 samples were 8 pmol or less while in 18 samples they ranged between 16 and 32 pmol, i.e. 18 (58.06%) samples showed resistance to chloroquine. All the 31 samples were further assessed by a probit analysis using the log-dose response test to know the degree of sensitivity and effective concentration (EC) from the grouped data. The drug concentrations and inhibition of schizont maturation (%) of sensitive and resistant isolates are given in Table 3. The effective concentrations of sensitive isolates, i.e. EC50 and EC90, were 0.28 and 0.96 µM/litre blood respectively. The 18 resistant isolates were divided according to their level of resistance. In three isolates, the upper limit of schizont maturation occurred at 3.2 µM/litre while in 15 isolates it occurred at 1.6 µM/litre blood. The average EC50 and EC90 in resistant isolates are 0.66 and 1.44 µM/litre blood (Table 3).

### *In-vivo* test

As many as 32 cases were successfully fol-

**Table 1. Incidence of malaria in Indian Oil Corporation, Mathura***

| Year | Total +ve | *Pv* | *Pf* | API |
|---|---|---|---|---|
| 1982 | 1809 | 1714 | 95 | 113.06 |
| 1983 | 1441 | 1315 | 126 | 90.06 |
| 1984 | 4593 | 3146 | 1447 | 287.06 |
| 1985 | 1270 | 1119 | 151 | 79.37 |
| 1986 | 1028 | 857 | 171 | 64.25 |
| 1987 | 602 | 370 | 232 | 37.62 |
| 1988 | 1027 | 694 | 333 | 64.18 |
| 1989 | 884 | 749 | 135 | 55.25 |
| 1990 | 669 | 483 | 186 | 41.81 |

* Total population : 16,000 (Source : Chief Medical Officer, Mathura Refinery Hospital).

**Table 2. *In-vivo* and *In-vitro** sensitivity of chloroquine to *P. falciparum***

| | Total No. of cases followed | | Sensitive | | | Resistant† | |
|---|---|---|---|---|---|---|---|
| *In-vivo* | | 32 | 19 (59.37%) | | | 13 (40.62%) | 12 (RI level)<br>1 (RII level) |
| | No. of isolates tested | Growth in control | MICs (pmol) | | | | |
| | | | 4 | 8 | 16 | 32 | % showing resistant |
| *In-vitro* | 38 | 31(81.57%) | 3 (9.68%) | 10 (32.25%) | 15(48.38%) | 3 (9.68%) | 58.06 |

*Considered valid when 10% schizont maturation observed in post-incubation control wells[7,8]; †Schizont maturation at 8 pmol of chloroquine considered resistant[10]. Out of 13 resistant cases, 12 were RI level resistants while one RII resistant.

lowed up to 28 days from a total of 36 cases. 13 cases were found resistant while 19 cases were sensitive to chloroquine (Table 2). Out of 13 resistant cases, 12 showed RI level of resistance where the parasite reappeared between D13 and D21, while one case was identified as RII level of resistance where parasitaemia was markedly reduced after the initiation of treatment but with no subsequent disappearance of asexual parasites after D6.

**Table 3. Analysis of chloroquine sensitivity (*in vitro*) of *P. falciparum* from Indian Oil Corporation, Mathura**

| Isolates in 1990 | No. of isolates | Drug concentration ($\times 10^{-6}$ mol/litre) and mean percentage inhibition of schizont maturation* | | | | | | Effective concentration | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.2 | 0.4 | 0.8 | 1.6 | 3.2 | 6.4 | EC50 | EC90 |
| Sensitive | 13 (41.93%) | 36.55 | 65.60 | 89.16 | 100 | 100 | 100 | 0.28 | 0.96 |
| Resistant† | 18 (58.06%) | 12.97 | 31.87 | 64.15 | 92.28 | 99.05 | 100 | 0.66 | 1.44 |

*Schizont maturation at (or above) a drug concentration (× 10 mol/litre blood)[11]; †Heterogeneity of parasite population from Thaithong[15] and Bruce-Chwatt *et al.*[14]; Out of 18 isolates, schizont maturation occurred in 15 at (or above) 1.6 μM/litre, and in 3 is at 3.2 μM/litre blood.

The parasite densities on D0, D2, D7 and D10 were 22,500, 11,250, 476 and 14,285 per mm blood respectively. All the resistant cases responded to metakelfin, and the parasites cleared within seven days of treatment. All 36 cases were also investigated for *in-vivo* response to chloroquine by the sentinel system. The parasite density in 35 cases on D2 was less than 25% of DO level, which showed that 35 cases were either sensitive or were of RI level of resistance while in one case, the parasitaemia on D2, was more than 25% of pretreatment count (D0) indicating RII level of resistance.

## DISCUSSION

Chloroquine resistance in *P. falciparum* is being recorded in the vicinity. This rules out the possibility that resistant foci got established owing to migration of itinerant labour from endemic areas. Hospital records showed that during the last eight years, i.e. 1982-83, 1983-84, 1984-85, 1985-86, 1986-87, 1987-88, 1988-89 and 1989-90, the consumption of chloroquine was 41,370, 148,772, 149,558, 140,263, 123,050, 82,500, 64,330 and 66,990 tablets (150 mg base) and 1590, 4580, 4870, 6220, 339, 2060, 950 and 1900 injections (5 ml) respectively, figures which show the indiscriminate use of antimalarials on the basis of clinical examination. Such indiscriminate use of chloroquine for several years coupled with improper treatment resulted in the selection of resistant strains[12].

Our study revealed a heterogeneity of parasite population[13] for different levels of the inhibition of schizont maturation indicating the development of incipient resistance[14]. Chloroquine resistance at IOC, Mathura, resulted mainly due to the indiscriminate use of antimalarials for several years which selected resistant stains[12]. Therefore, continuing medical education to medical personnel at the industrial complexes is necessary since malaria is often missed or misdiagnosed, and invariably treatment is inadequate or incomplete, which may otherwise precipitate early resistance in *P. falciparum* involving large areas from different geographical areas of India[15]. However, most of the studies were confined either to rural or urban areas and not much information is available about the foci of chloroquine resistance in industrial complexes except a few studies, i.e. National Thermal Power Corporation (NPTC), Shaktinagar; Vishakhapatnam Steel Project (VSP), Vishakhapatnam; Dandakaranya (DNK) Project, Bastar; and Bharat Heavy Electricals Limited (BHEL), Hardwar.

Our findings show the presence of chloroquine resistance in *P. falciparum* in IOC, Mathura. This area was surveyed in 1978 and no resistance in *P. falciparum* to chloroquine[5] was found, while our study showed that 50% of the cases were chloroquine-resistant. The probit analysis of the log-dose concentrations at which schizont maturation occurred indicates that 15 cases were of low level resistance (MIC, 1.6 µM/litre blood) while the remaining 3 had shown high level of resistance (MIC 3.2 µM/litre blood). Moreover, 7 out of 13 sensitive cases were in the proximity of resistance level (1 µM/litre blood)[16]. Similar results were obtained with *in-vivo* tests in which one case with RII level of resistance was found. Good correlation was

found between *in-vivo* 28-day extended test and *in-vivo* 7-day sentinel system.

Investigation of all resistant cases showed that most of them were indigenous and belonged to IOC complex or its vicinity.

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# Effect of Temperature on Toxicity of Two Bioinsecticides Spherix (*Bacillus sphaericus*) and Bactoculicide (*Bacillus thuringiensis*) against Larvae of Four Vector Mosquitoes

P.K. MITTAL*, T. ADAK* and V.P. SHARMA†

Two bioinsecticide preparations, viz. Spherix (*Bacillus sphaericus*) and Bactoculicide (*Bacillus thuringiensis* H-14), were tested in the laboratory against larvae of *Anopheles culicifacies, Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* at different temperatures. The $LCs_{50}$ of Spherix against III instar larvae of these species at 27±2°C were 2.0, 0.19, 0.05 and >40 mg/litre, respectively and those of Bactoculicide were 0.32, 0.16, 0.06 and 0.03 mg/litre, respectively. The toxicity of two bioinsecticides, especially Spherix, varied to a great extent when the tests were repeated at 21±2°C and 31±2°C. At 21±2°C, Spherix was almost non-toxic against larvae of *An. culicifacies* and *An. stephensi* ($LC_{50}$ >10 mg/l) but at 31±2°C the bioinsecticide was highly toxic against *An. culicifacies* ($LC_{50}$ = 0.48 mg/litre) and *An. stephensi* ($LC_{50}$ = 0.04 mg/litre). A similar effect of the temperature was also observed with Bactoculicide.

**Key words:** *Bacillus*, Biological control, Mosquito larvae, Toxicity

## INTRODUCTION

Bioinsecticide preparations based on *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* have been reported to produce effective control of mosquito larvae[1-3]. The two microbial agents also do not cause any harmful side effects on other non-target organisms[4,5]. The larvicidal activity of these microbial agents differs from strain to strain and also from

* Malaria Research Centre, 2, Nanak Enclave, Delhi-110 009, India.
† Malaria Research Centre, 20, Madhuvan, Delhi-110 092, India.

species to species of mosquitoes[6,7]. In addition, the efficacy of *Bacillus thuringiensis* and *Bacillus sphaericus* preparations depends on fermentation and formulation methods and also on environmental factors[8,9]. Temperature is one of the important factors which can influence the larvicidal activity of these bioinsecticide agents to a great extent[10,11]. This study deals with the comparative toxicity of two bioinsecticides, viz. Spherix (a formulation of *Bacillus sphaericus*) and Bactoculicide (a formulation of *Bacillus thuringiensis* H-14), against larvae of *Anopheles culicifacies* and *Anopheles stephensi*, the vectors of malaria in rural and urban areas. *Culex quinquefasciatus* is a vector of bancroftian filariasis and a pest mosquito, and *Aedes aegypti*, is the vector of dengue fever. The effect of temperature on their larvicidal activity has also been studied.

## MATERIALS AND METHODS

Spherix (*B. sphaericus*) and Bactoculicide (*B. thuringiensis* H-14) used in this study were Russian products, supplied by Chemicals International Limited, Delhi. The mosquito larvae of *An. culicifaces, An. stephensi, Culex quinquefasciatus* and *Ae. aegypti* were obtained from the insectary at Malaria Research Centre (MRC). To determine the toxicity of bioinsecticides against different mosquito species, the bioassay tests were carried out at 27±2°C, using 25 larvae of the test species in 250 ml of stored water in plastic bowls. Only late III instar larvae were used for the bioassay tests. The larvae were exposed to serially diluted concentrations of the bioinsecticide suspension in water, which were made immediately prior to bioassay by thoroughly shaking the biocide suspension on a magnetic stirrer. Each test was replicated four times at 5 different concentrations along with a control and the corrected per cent mortality was determined by using Abbott's formula:

$$\% \text{ mortality} = \frac{\left(\begin{matrix}\% \text{ mortality in} \\ \text{the experiment}\end{matrix}\right) - \left(\begin{matrix}\% \text{ mortality} \\ \text{in control}\end{matrix}\right)}{100 - (\% \text{ mortality in control})} \times 100$$

The lethal concentrations for 50% and 90% mortality ($LC_{50}$ and $LC_{90}$) were determined by the dosage-mortality regression line plotted on log-probit paper and the confidence limit of $LC_{50}$ with a probability of 95% were calculated by the method of Litchfield and Wilcoxon[12]. To study the effect of temperature on larvicidal activity of the two bioinseciticides, each test was repeated at two different temperatures (21±2°C and 31±2°C) for which the bowls were placed in a B.O.D. incubator set at the required temperature.

## RESULTS AND DISCUSSION

The comparative toxicities of Spherix and Bactoculicide against larvae of *An. culicifacies, An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* are given in Table 1. Spherix was most effective against larvae of *Culex quinquefasciatus* ($LC_{50}$ = 0.056 mg/litre) followed by *An. stephensi* ($LC_{50}$ = 0.19 mg/litre) and *An. culicifacies* ($LC_{50}$ = 2.0 mg/litre). *Aedes aegypti* was, however, not susceptible to Spherix ($LC_{50}$ > 40 mg/litre). Bactoculicide was most effective against *Ae. aegypti* ($LC_{50}$ = 0.034 mg/litre)

**Table 1. Comparative toxicities of Spherix and Bactoculicide against III instar larvae of mosquitoes**

| Mosquito spp. | LC | Concentrations (mg/litre) and 95% confidence limits after 40 h exposure at 27±1°C | |
|---|---|---|---|
| | | Spherix | Bactoculicide |
| *An. culicifacies* | $LC_{50}$ | 2.0 (1.635-2.446) | 0.32 (0.26-0.39) |
| | $LC_{90}$ | 7.8 | 1.1 |
| *An. stephensi* | $LC_{50}$ | 0.19 (0.135-0.239) | 0.16 (0.123-0.207) |
| | $LC_{90}$ | 0.82 | 0.92 |
| *Cx. quinquefasciatus* | $LC_{50}$ | 0.056 (0.046-0.067) | 0.062 (0.049-0.078) |
| | $LC_{90}$ | 0.22 | 0.27 |
| *Ae. aegypti* | $LC_{50}$ | >40- | 0.034 (0.026-0.043) |
| | $LC_{90}$ | — | 0.098 |

followed by *Culex quinquefasciatus* ($LC_{50}$ = 0.062 mg/litre), *Anopheles stephensi* ($LC_{50}$ = 0.16 mg/litre) and *An. culicifacies* ($LC_{50}$ = 0.32 mg/litre). The susceptibility of *Culex quinquefasciatus* against Spherix was even higher than against Bactoculicide. Both the preparations were effective against two anopheline species, the important vectors of malaria in India.

*Bacillus sphaericus* formulations have been reported to be highly insecticidal against larvae of *Culex* and certain species of *Anopheles* but not against *Aedes aegypti* [6]. The toxicity of *Bacillus sphaericus*, particularly against larvae of *Anopheles* species, is generally low as compared to that of *Bacillus thuringiensis* H-14, and the mortality is obtained in 48 to 72 h[13]. But it has been reported to persist and recycle in the treated habitats[14,15].

The larvicidal activity of the two bioinsecticides, especially Spherix, showed much variation, when tested at different temperatures (Table 2). At 21±2°C Spherix was almost nontoxic against *Anopheles culicifacies* and *Aedes aegypti* ($LC_{50}$ > 40 mg/litre) and much less toxic against *An. stephensi* ($LC_{50}$ > 10 mg/litre); however, the same formulation at a higher temperature, viz. 31±2°C, proved to be highly toxic against *An. culicifacies* ($LC_{50}$ = 0.48 mg/litre) and *An. stephensi* ($LC_{50}$ = 0.04 mg/litre), but the toxicity against *Aedes aegypti* showed only a slight increase with increase in temperature. A similar trend was also observed with Bactoculicide (Table 2). A direct relationship between temperature and the larvicidal activity of *B. thuringiensis* and *B. sphaericus* against *Culex quinquefasciatus* and *Aedes stimulans* larvae has also been reported[10,16].

**Table 2. Effect of temperature on larvicidal activity of Spherix and Bactoculicide against different vector species**

| Bioinsecticide | Mosquito spp. | $LC_{50}$ values (mg/litre) with 95% confidence limits after 40 h exposure at two temperatures | |
|---|---|---|---|
| | | 21°C | 31°C |
| Spherix | *An. culicifacies* | >48- | 0.48 (0.39-0.59) |
| | *An. stephensi* | >10*- | 0.04 (0.033-0.056) |
| | *Cx. quinquefasciatus* | 0.088 (0.072-0.106) | 0.032 (0.026-0.39) |
| | *Ae. aegypti* | >40- | >10*- |
| Bactoculicide | *An. culicifacies* | 0.8 (0.635-1.01) | 0.17 (0.13-0.22) |
| | *An. stephensi* | 0.25 (0.192-0.324) | 0.076 (0.061-0.094) |
| | *Cx. quinquefasciatus* | 0.19 (0.156-0.230) | 0.022 (0.017-0.027) |
| | *Ae. aegypti* | 0.07 (0.56-0.088) | 0.021 (0.018-0.025) |

* $LC_{50}$ for Spherix against *An. stephensi* at 21°C was roughly 10.2 mg/litre and against *Ae. aegypti* at 31°C was roughly 32 mg/litre respectively.

The increased larvicidal activity at higher temperatures may probably be due to the increased metabolic activity of the larvae, resulting in rapid uptake or activation of bioinsecticide toxins in the larval gut. Many *Bacillus sphaericus* formulations which show delayed activity against anopheline larvae and appear to be almost nontoxic against *An. culicifacies* may prove effective at higher ambient temperatures.

The results of this study show the high toxicity of the two Russian bioinsecticides against larvae of major vector mosquitoes, particularly *An. culicifacies* and *An. stephensi*. Further, the toxicity of these bioinsecticides was temperature-dependent and much higher toxicity was observed at higher temperatures, which is also suitable for the profuse breeding of mosquitoes in the field. In the light of these observations the two bioinsecticides may prove to be highly effective under tropical climates. Further studies are in progress on the efficacy of these bioinsecticides in the control of vector breeding and interruption of transmission in field conditions.

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# SHORT NOTE

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# Complication of Intramuscular Quinine Injection: Three Case Reports

R.SATPATHY*, D.B.DAS*, N.C.NANDA*, J.K.PATNAIK#, S.K.MISHRA† and B.S.DAS‡

**Key words:** Chemotherapy, Clinical malaria, Quinine complications

Quinine is frequently used in intramuscular (IM) or intravenous (IV) route in the treatment of malaria[1] in endemic areas. Intramuscular use is discouraged as it causes massive local tissue necrosis. Though unusual, a few reports in literature suggest that gluteal IM injection (phenobarbitone, analgesics, sulfonamides, antibiotics like penicillin, and vitamin K) can inadvertently be delivered in to gluteal arteries or their branches or can directly injure the sciatic nerve[1] resulting in distal ischaemia, gangrene and/or neurologic deficits[2,3]. We report three such cases of unusual complications following IM quinine injection in children.

*Case No. 1:* N.A., 7-year boy, was referred from a mines area hospital in the District of Sundergarh. The boy had complaints of severe pain, inability to walk and discoloration of the toes of right lower limb following injection. History revealed that as a case of M.T. malaria he was administered IM quinine injection to the right buttock 12 days before. Soon after he experienced severe burning and tingling pain over the right leg which continued for the whole day. The next day, parents noticed discoloration of all the toes of the right leg and inability to move the limb. Examination (12th day) showed: temp.- 102°F, pulse - 106/m felt in all limbs, and B.P.

* Department of Paediatrics; #Department of Pathology; †Department of Internal Medicine; ‡Department of Biochemistry, Ispat General Hospital, Rourkela-769 005, India.

*Fig. 1:* Gangrene of right toes.

- 110/60 mm Hg. He was thin, pale, limping to right and had splenomegaly. Examination of nervous system showed normal higher functions and no cranial nerve deficit. Motor functions—muscular wasting was seen in thigh and calf muscles of the right lower limb with tenderness and hypotonia. Muscle power in right lower limb : Gr - 0/5 in peroneal group and Gr - 3/5 in quadriceps and hamstrings with foot drop. Sensory examination revealed loss of sensation over dorsum of right foot and sole extending posterolaterally up to leg. All superficial reflexes including plantar were normal. Ankle jerk was absent and knee jerk was depressed in the right lower limb. No peroneal nerve thickening was felt. The tips of the toes were coppery red (Fig. 1). The redness extended to 1 cm proximal to tip of toes, and the great toe was least involved. There was no local tissue necrosis at the site of injection. Other systems were normal. Hb was 8.0 g/dl and M.T. and B.T. rings were found in peripheral blood. Fasting blood sugar and post-prandial blood sugar were 86 mg/dl and 94 mg/dl respectively. Urine was free of sugar. Doppler confirmation of the vascular obstruction could not be done as the facility was not available. He was treated with oral chloroquine and primaquine. Sloughing of toes was noticed (17th day) and Betadine dressing was continued. He was discharged (40th day) with advice to take care of the foot and physiotherapy. Follow-up after 6 months showed sloughing and healing in all toes with persistent neurological deficit.

*Case No. 2:* R.N., 5-year girl, referred from the same area (as in Case No. 1). She was brought to hospital with complaints of pain and numbness in the left foot and limping to left for the last 7 days. The symptoms followed 2 days after the IM injection of 3 doses of quinine given on both buttocks. Records revealed that she had suffered from malaria on four occasions in the last three years. She had been immunized fully against poliomyelitis. On examination (9th day) she was 14 kg, pale, of average build and was limping to left. Pulse was 86/m, felt in all limbs, BP was 100/64 mm Hg and there was splenomegaly. Examination of the nervous system showed normal higher functions and no cranial nerve deficit. Motor functions showed no wastings of any group of muscles, power Gr-0/5 in peroneal muscles, Gr-4/5 in hamstrings and quadriceps in left lower limb with foot drop. Sensory examination revealed impaired pain and touch sensation all over the toes and lateral side of the left foot. All the superficial reflexes including plantars were normal. Left ankle jerk was absent. There was no peripheral nerve thickening. Fundus examination was also normal. There was no tissue necrosis at the site of injection. Investigation showed Hb-9.5 g/dl. Blood film was negative for M.P. Lumbar tap done showed C.S.F.- 2 cells/hpf, protein - 40 mg/dl, and sugar - 60 mg/dl. The case was discharged (18th day) with advice of physiotherapy and vitamins. The follow-up after 3 months showed no improvement in neurologic deficit.

*Case No. 3:* SKM, 14-year boy, from mines area in the district of Keonjhar, was brought with complaints of limping to right for the last 3 months, pain in right calf, and right anterior portion of sole. The symptoms started after one injection of IM quinine given on the right buttock. There was immediate tingling sensation, numbness and pain, all started from the site of injection to foot. Subsequently all injections were stopped and the boy was observed for 2 months. Limp, which was worst from 5th to 16th day, slowly improved but persisted. Examination (three months after) showed: wt - 27 kg, thin built, B.P. - 110/70 mm Hg, and no splenomegaly. Spine was normal. Limp was towards right. Nervous system examination showed higher functions and cranial nerves were normal. Motor functions revealed wasting of calf and thigh muscles of the right lower limb without foot drop and power Gr - 5/5 in all groups of muscles. All modalities of sensations were depressed in L-5 and S-1 segments. X-ray spine was also normal. TLC 11,000/dl, Hb - 11.0 g/dl, MP -ve, PPD -ve. The boy was put on $B_1$, $B_6$, $B_{12}$ vitamins and physiotherapy. He was discharged after a week of hospitalization.

With only 4% of India's population, Orissa contributes to 48% of total malarial deaths and 31% of falciparum cases[4]. The three reported cases are from the mines area where malaria is endemic with chloroquine resistance[5]. Many children are treated with oral chloroquine and quinine IM injections several times a year and as such are treated at home. IV administration of antimalarial drugs is time-consuming and needs hospital stay which is most often not possible because of the large number of cases and inadequate indoor facility.

All the three children were given IM quinine in a dose 10 mg/kg of body weight as they were seriously ill, refusing oral feeds, and belonged to chloroquine-resistant area[5]. Quinine dihydrochloride was used. The diagnosis of drug-induced neuropathy is based on exclusion of diseases like diabetes, poliomyelitis, leprosy and myelopathies, and by taking into account the age, immunization status and other relevant clinical findings.

In most cases of extremity gangrene in children a discussion of aetiology is largely a statement of ignorance as to indicating factors[6]. However, the various factors are thermal, inadvertent arterial infusion of intramuscular drugs, pH and osmolality of infusate, etc. including direct sciatic nerve injury. Accidental intra-arterial injection following intramuscular penicillin to buttock has been described by Knowles[3], causing vascular occlusion and leg paralysis. IM injection of vitamin K to a newborn's buttock resulting in gangrene of foot and another case of a one and half year child developing gangrene of one hand following injection into deltoid have been reported by Dhar *et al*[2]. Three cases of lower limb gangrene and paralysis following umbilical cord injection have been described[6].

The possible mechanism in the first case is a misplaced IM injection in buttock causing trauma to both tibial and peroneal divisions of sciatic nerve and accidental injection into the accompanying vessel causing vasospasm, thrombosis and ischaemia distally. The gangrenous involvement of the toes could be due to forceful IM injection resulting in retrograde flow of medication to common illiac and down into the external illiac artery[3]. Occlusion of sciatic artery, a branch of inferior gluteal artery, by direct IM injection has also been postulated. In the second and third cases, misplaced IM injections in buttock injured the sciatic nerve causing foot drop and sensory loss. Peroneal division of the sciatic nerve is six times more likely to be damaged than the tibial division causing more of foot drop and walking difficulties than marked sensory loss[7].

The injury occurring in all cases did not show satisfactory recovery of neurological as well as the vascular symptoms during a follow-up period of 3 to 9 months. As quinine is a powerful local anaesthetic, the anaesthesia produced by such injection near a peripheral nerve may last for weeks or months[8]. Therefore, it is suggested that IM quinine injections should be given carefully to anterior thigh with strict aseptic technique[1] only when it cannot be avoided.

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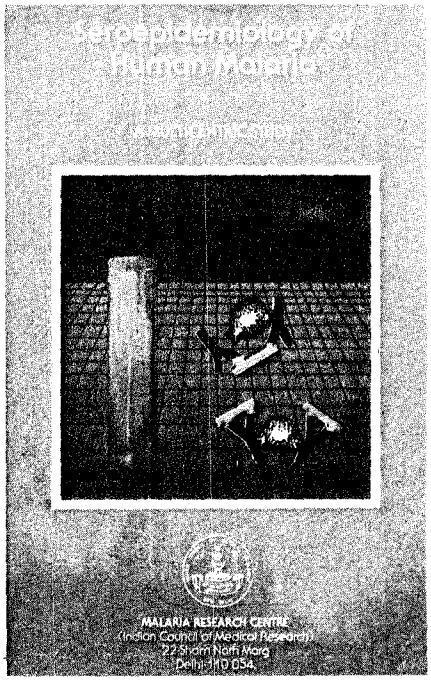

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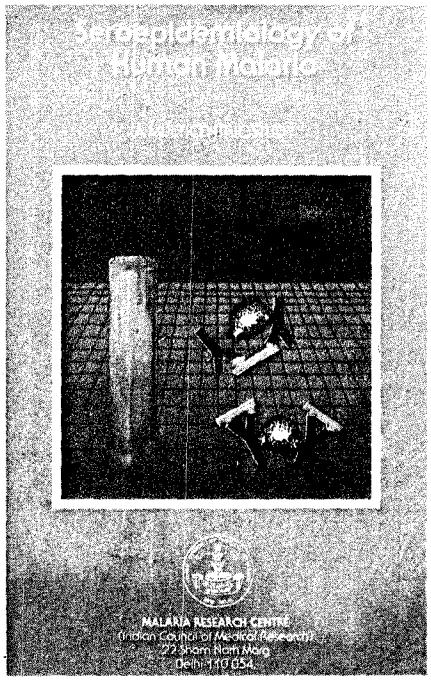

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