EFFECT OF DDT ON SURVIVAL AND BLOOD FEEDING SUCCESS OF ANOPHELES ARABIENSIS IN NORTHERN KWAZULU, REPUBLIC OF SOUTH AFRICA

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ABSTRACT. The effect of house spraying with DDT on blood-feeding and resting behavior of An. arabiensis in Natal Province, Republic of South Africa, was investigated. Indoor resting occurred in both control (unsprayed) and replastered (DDT on walls covered due to replastering) huts, but was minimal in fully DDT-sprayed huts. The percentage of bloodfed mosquitoes was >50% in both control and replastered huts, but in the latter huts there was a reduction in the percentage of gravid and an increase in the percentage of unfed mosquitoes. Large numbers were collected in exit traps irrespective of the DDT status of the huts. The percentage of catch that was bloodfed was lowest in exit traps fitted on DDT-sprayed houses. The percentage survival of bloodfed mosquitoes caught in exit traps exceeded 72% for all 3 hut types. The survival of unfed mosquitoes was, however, markedly lower. Susceptibility tests showed no physiological tolerance to DDT by the wild An. arabiensis population. Bioassays using colonized An. arabiensis showed the DDT deposits on hut walls to effect 100% kill with 15 minutes of exposure.

INTRODUCTION

The efficient control of vector species is central to the containment of malaria. The development of low cost residual insecticides after World War II revolutionized rural malaria control (Davidson 1982). The use of intra-domiciliary insecticides for malaria vector control has, however, met with variable success depending on logistical and social factors, finance, vector behavior and the development of insecticide resistance (Fontaine 1983). It still, however, remains an important component of malaria control in some rural areas (Gratz 1985). The assessment of the efficiency of this measure in an area is essential to planning an effective malaria control strategy.

Three species of the Anopheles gambiae Giles complex, An. quadriannulatus Theobald, An. arabiensis Patton and An. merus Donitz occur in Kwazulu (le Sueur and Sharp 1988), an area experiencing seasonal malaria transmission (Sharp et al. 1988). The malaria case rate has increased over the past 2 years, in part due to chloroquine resistance in Plasmodium falciparum Welch (Freese et al. 1988).

This study investigated intra-domiciliary DDT vector control in the malaria area of northern Kwazulu, Republic of South Africa.

MATERIALS AND METHODS

Specimens for the study were collected at Mamfene (36° 16’ E, 27° 23’ S), Ubombo district during the period January 1986 to April 1988.

Exit traps (Muirhead-Thomson 1947) were used and emptied before 0800 h each day. Indoor resting catches were made by space spraying with a 4% pyrethrum and paraffin solution using a Rega pump.

After capture, the mosquitoes to be used for susceptibility testing were bloodfed, placed individually in breeding tubes to oviposit and held in an insectary (25 ± 1°C, 75 ± 5% RH). The breeding tubes were 25-ml plastic bottles with damp cotton wool covered by filter paper on the bottom and with gauze tops. Individual family broods were reared. Bioassay tests (World Health Organization 1975) were performed using 1- to 4-day-old adult females.

After removal from the exit traps, the mosquitoes were stored in collecting cups in an insulated container covered with damp muslin cloth. Mortality counts were done periodically until 12-16 h after collection (0800 h), and the gonotrophic status of both the surviving and dead mosquitoes was scored.

Species identification of the An. gambiae s.l. was by polytene chromosome analysis (Coluzzi 1968, Hunt 1973, Green and Hunt 1980) and isozyme electrophoresis (Mahon et al. 1976; Miles 1978, 1979).

Contact bioassays (World Health Organization 1975) were done on the DDT-sprayed walls of houses using 2- to 5-day-old An. arabiensis from a colony strain (KANB) that originated from Kanyemba, Zimbabwe (15°40’ S, 30°20’E). A modification to the technique was in the use of shorter exposure times as well as the standard 1-h exposure.

RESULTS

Seventy-nine An. arabiensis were identified by chromosome analysis, 65 caught by exit trap
and 14 by indoor resting collections. Sixty-five
were identified by both chromosomes and elec-
trophoresis. All 65 specimens identified by chro-
osomes had the following rf values: superoxide
dimutase (SOD, E.C. 1.15.1.1.) 100/100; aspar-
tate aminotransferase (AAT, E.C.2.6.1.1.) 100/
100; and octogonal dehydrogenase (ODH, E.C.
1.1.1.73) 95/95. A further 559 specimens were
identified by electrophoresis (Table 1). Identifi-
cation of indoor resting and exit trap caught
An. gambiae s.l. showed >96% to be An. arabiensis
with very low numbers of An. quadriannulatus
present (Table 1).

Both exit trap and indoor resting catches were
successfully used to collect An. gambiae s.l.
(Tables 2 and 3). The mean number caught per
hut was higher in exit trap collections than in
the indoor resting collections, but this difference
was not significantly different (t = 1.608, P :
0.108). Anopheles gambiae s.l. were caught leav-
ing huts irrespective of the presence or absence
of DDT (Table 2). There was, however, an in-
verse relationship between the presence of DDT
and the numbers of An. gambiae s.l. caught, with
the highest numbers per hut being caught in
control huts. The range in numbers caught was
high irrespective of hut status, and pairwise
comparisons with a Mann-Whitney test showed
no significant difference between catches from
huts of differing DDT status (P > 0.5).

Indoor resting occurred mainly in replastered
and control huts (Table 3). Only 7 An. gambine
s.l. were caught resting indoors in a total of 79
DDT-sprayed huts, and 6 of these were caught
in one hut. The Mann-Whitney test showed no
significant difference between catches from con-
trol and replastered huts (P > 0.7), but in both
cases these data were significantly different to
that from the DDT-sprayed huts (P < 0.003).

A greater percentage of the An. gambiae s.l.
cought leaving huts (Table 4) were unfed, fol-
lowed by freshly fed and lastly a low number of
gravid mosquitoes. There was an inverse rela-
tionship between the percentage of freshly
blooded mosquitoes and the presence of DDT.
The highest percentage of freshly blooded spec-
imens were caught leaving control huts, while
an increased proportion of unfed mosquitoes left
the DDT-sprayed huts (Table 4). The percent-
age of gravid females caught in all huts was
extremely low. Chi-square tests (2 x 3) of het-
erogeneity showed that the gonotrophic status
of An. gambiae s.l. from the different hut cate-
gories were significantly different (P < 0.001).

The majority of An. gambiae s.l. caught resting
indoors were freshly blooded, followed by
unfeds, with the lowest numbers being in the
gravid state (Table 5). The percentage blooded
was high irrespective of the DDT status of the
hut. The percentage gravid was higher than the
percentage unfed in both the DDT-sprayed and
the control huts. Only 6 An. gambine s.l. were
cought in the DDT-sprayed huts. In the replas-
tered huts the percentage unfed was greater than
the percentage gravid. The Mann-Whitney test
showed no significant difference between the
total numbers of An. gambine s.l. caught in the
control and replastered huts (P > 0.7), but a
Chi-square test of heterogeneity showed the
gonotrophic status of the mosquitoes from the 2
hut types to be significantly different (P < 0.005). Data collected from the DDT-sprayed
huts were excluded from statistical analysis due
to the low numbers caught (n = 6).

Survival of exit trap caught mosquitoes was
highest in the control huts, less in the replas-

| Table 1. Identification of Anopheles gambiae s.l. by electrophoresis from exit traps and indoor resting collections. |
|----------------|----------------|----------------|
| Trap           | An. arabiensis | An. quadriannulatus |
| Exit trap      | 301            | 12              |
| Indoor resting | 236            | 10              |
| Total          | 537            | 22              |

| Table 2. Exit trap caught Anopheles gambiae s.l. in control, replastered and DDT sprayed huts. |
|----------------------------------|----------------|----------------|----------------|----------------|
| No. huts                        | 60             | 150            | 155            | 365            |
| No. An. gambiae s.l.             | 252            | 512            | 312            | 1076           |
| \( \bar{x} \pm \text{SE/hut} \)  | 4.2 ± 1.3      | 3.4 ± 0.7      | 2.0 ± 0.5      | 2.94 ± 0.40    |
| Range                           | 0–59           | 0–86           | 0–68           | 0–86           |

| Table 3. Indoor resting Anopheles gambiae s.l. in control, replastered and DDT sprayed huts. |
|----------------------------------|----------------|----------------|----------------|----------------|
| No. huts                        | 23             | 57             | 79             | 159            |
| No. An. gambiae s.l.             | 68             | 213            | 7              | 288            |
| \( \bar{x} \pm \text{SE per hut} \) | 2.9 ± 1.0      | 3.7 ± 1.5      | 0.1 ± 0.1      | 1.9 ± 0.5      |
| Range                           | 0–17           | 0–69           | 0–6            | 0–69           |
Table 4. Gonotrophic status of *Anopheles gambiae* s.l. from exit traps.

<table>
<thead>
<tr>
<th></th>
<th>% bloodfed</th>
<th>% gravid</th>
<th>% unfed</th>
<th>No. <em>An. gambiae</em> s.l.</th>
<th>No. huts</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>32.6</td>
<td>0.3</td>
<td>67.1</td>
<td>310</td>
<td>153</td>
</tr>
<tr>
<td>Replastered</td>
<td>41.9</td>
<td>5.5</td>
<td>52.5</td>
<td>489</td>
<td>146</td>
</tr>
<tr>
<td>Control</td>
<td>65.5</td>
<td>2.5</td>
<td>32.0</td>
<td>237</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>44.7</td>
<td>3.4</td>
<td>51.9</td>
<td>1,036</td>
<td>357</td>
</tr>
</tbody>
</table>

Table 5. Gonotrophic status of indoor resting *Anopheles gambiae* s.l.

<table>
<thead>
<tr>
<th></th>
<th>% bloodfed</th>
<th>% gravid</th>
<th>% unfed</th>
<th>No. <em>An. gambiae</em> s.l.</th>
<th>No. huts</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>66.6</td>
<td>33.3</td>
<td>0.0</td>
<td>6</td>
<td>78</td>
</tr>
<tr>
<td>Replastered</td>
<td>61.5</td>
<td>13.8</td>
<td>24.6</td>
<td>195</td>
<td>53</td>
</tr>
<tr>
<td>Control</td>
<td>52.0</td>
<td>36.0</td>
<td>12.0</td>
<td>50</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>61.0</td>
<td>18.9</td>
<td>20.0</td>
<td>251</td>
<td>150</td>
</tr>
</tbody>
</table>

 Twenty-five and 17 colonized female *An. arabiensis* were used, respectively, in 0.5- and 1.0-h control bioassays in non-DDT-sprayed huts, as part of the 3-month post-DDT spraying bioassays, and 20 and 36, respectively, in 10- and 30-min bioassays as part of the 9-month post-spraying bioassays. There was no mortality in any of the control bioassays. Five (*n* = 54), 10 (*n* = 53) and 30 min (*n* = 67) of contact on 3-month-old DDT was sufficient for 100% kill in all cases. In the case of 9-month-old DDT, 5 min of contact only effected 26% mortality (*n* = 19); however, 15 min or more (*n* = 54) of contact effected 100% mortality.

Families raised from 21 individually identified *An. arabiensis* totaling 339 individual mosquitoes were tested for DDT susceptibility. These mosquitoes and samples of colonized *An. arabiensis* were respectively exposed to 5 standard concentrations of DDT for 1-h and the 24-h mortality plotted on logarithmic probability paper to construct dosage mortality regressions (Fig. 1). Neither regression indicates insecticide resistance in the populations concerned at a discriminating dosage of 4.0% DDT. The figures indicate that the wild population (Fig. 1) had increased vigor tolerance relative to the colonized population. In the colonized population, 100% mortality was obtained at a concentration of 2.0% DDT, whereas in the wild population, this occurred at a 4.0% DDT concentration.

**DISCUSSION**

The diagnostic allozymes of chromosomally identified *An. arabiensis* correlated well with the results of Miles (1979) and Marchand and Mnzava (1985) for specimens from East Africa. In this study the specimens showed no obvious polymorphism for the AAT and SOD isoenzyme system, and all ODH rf values were monomorphic (95/95). These data validate the use of the Miles (1979) key for the electrophoretic identification of *An. arabiensis* from this area.

*Anopheles arabiensis* and *A. quadriannulatus* were caught both resting indoors and leaving huts in the study area. However, *An. arabiensis* was the dominant species, accounting for >96% of the identified specimens. As a consequence, these data are considered to reflect the behavior of *An. arabiensis*.

Overall, a higher number were caught leaving huts than resting indoors. However, a direct relationship did exist between the numbers caught leaving and the level of DDT. These data indicate the expected, with the highest numbers being caught in the control hut, a reduced number in the replastered huts where the underside of the roof had exposed DDT and the lowest number in the fully DDT-sprayed huts. Extremely low numbers were found resting in DDT-sprayed huts: of 79 huts investigated, only 2 were positive. Indoor resting occurred com-
Table 6. Percentage survival and percentage bloodfed of *Anopheles gambiae* s.l. from exit traps in relation to DDT status of hut.

<table>
<thead>
<tr>
<th>Status of hut</th>
<th>Number</th>
<th>% bloodfed</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Huts</td>
<td>Mosquitoes</td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>49</td>
<td>222</td>
<td>31.5</td>
</tr>
<tr>
<td>Replastered</td>
<td>13</td>
<td>91</td>
<td>53.8</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>82</td>
<td>86.6</td>
</tr>
<tr>
<td>DDT</td>
<td>6</td>
<td>65</td>
<td>26.2</td>
</tr>
<tr>
<td>Replastered</td>
<td>21</td>
<td>119</td>
<td>54.6</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>82</td>
<td>86.6</td>
</tr>
</tbody>
</table>

*1-3 months after DDT application.  
2-12 months after spraying.

The fed:gravid ratio varied tremendously between trap types and within trap types depending on the DDT status of the hut. The indoor resting ratios from control (1.4:1) and replastered (4.5:1) huts were similar to those found for *An. arabiensis* resting in DDT-sprayed huts (3.7:1) and outdoors (2.2:1) (Haridi 1972) and by Shelley (1973) for indoor resting collections in control huts (3.9:1). However, in the replastered huts there was an increase in the bloodfed component of the ratio. This ratio was markedly different in the exit trap caught mosquitoes, with a marked increase in the percentage bloodfed (control huts = 25.8:1 and replastered huts = 7.9:1) and particularly so in the case of DDT-sprayed huts (101:1). From these data it is clear that a high percentage of bloodfed mosquitoes were leaving the huts and not resting indoors. This tendency was increased in the fully DDT-sprayed huts and could be related to the irritational effect of DDT (Muirhead-Thomson 1960).

The survival of mosquitoes caught leaving huts 1-3 months after DDT spraying was not markedly different from that 8-12 months after application. Survival varied depending on the gonotrophic status of the mosquito and in relation to the DDT status of the hut. The percentage of bloodfed mosquitoes showed an inverse relationship to the level of DDT and suggests that the presence of DDT hampered the taking of a bloodmeal. The percentage survival of exit trap caught bloodfed mosquitoes from DDT-sprayed huts (72.9%, 1-3 months after application, and 88.2%, 8-12 months after spraying) compares well with similarly treated data collected by Haridi (1972) who found an 88% survival. The increased survival of engorged females is further in agreement with the findings of Trapido (1954). The survival of engorged *An. arabiensis* in this study were far higher than that commonly in both replastered and control huts. The indications from this are that the mosquitoes were resting on the DDT-free walls of the replastered huts. Had they been mainly resting on the underside of the roof, then comparable results would have been expected from both the DDT and replastered huts; the lack of physiological tolerance to DDT further precludes roof resting. These data are in contrast to that of Brun (1973) from Burkina Faso where in excess of 70% of the indoor resting *An. gambiae* s.l. were on the roof.

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found by Mpofo et al. (1988). They recorded 4% survival 3 months after DDT spraying and 30% survival at 8 months post-spraying, in contrast to the 72.9 and 88.2% found in this study. The reason for the higher survival recorded in this study can in part be attributed to the method of calculation of percentage survival and the mosquitoes used. In this study, only exit trap caught mosquitoes were used in this calculation, whereas Mpofo et al. (1988) used both exit trap and hut floor mortalities in their calculation. The mosquitoes used by Mpofo et al. (1988) were derived from a colony strain, whereas in this study, wild mosquitoes were used. Susceptibility tests done on both material from the wild population and colony-derived material showed the wild population to have increased DDT vigor tolerance relative to the laboratory colony material. Overall, survival of exit trap catches (all gonotrophic stages) in the study by Haridi (1972) was 94% and far higher than the 44.1 and 50.8% of this study. It is, however, expected that the proportion leaving and surviving will vary considerably according to the dosage, coverage, formulation, nature of the wall surface and behavior of a particular species (Muirhead-Thomson 1950, Hadaway 1951, Wilkinson 1952, Haridi 1972). The percentage mortality in this study is considered to be exaggerated as a result of high mortality in the unfed component of the exit trap catches. Unfed mosquitoes collected in exit traps from control huts only showed a 54.6 and 54.5% survival. Overall, survival of exit trap caught An. arabiensis showed an inverse relationship to the presence of DDT, with the lowest survival (44.1%) from the DDT-sprayed huts 1–3 months after spraying. If the mortalities of the unfed component of the exit trap catches are corrected using Abbott’s formula, bearing in mind the limitation of using this correction with such a high mortality in the control, mortality of unfed mosquitoes caught by exit traps on replastered and DDT huts, respectively, was as low as 30.2 and 43.4%.

Bioassays in DDT-sprayed huts indicate minimal loss of effectiveness of the DDT deposits over time in killing An. arabiensis. In fact, 8–12 months after application an exposure time of 15 min was sufficient to effect 100% mortality. Dose mortality curves further showed no marked increased vigor tolerance or physiological resistance to DDT in the wild An. arabiensis relative to WHO standards, with 100% mortality occurring at a discriminating dosage of 4% DDT. Overall, a higher percentage of An. arabiensis were leaving huts than were resting inside. The mean number caught leaving replastered huts compared to control huts was reduced by 19% and in DDT huts by 52%. Assuming random entry of huts, this reduction in exit trap catch is taken to represent the numbers killed indoors. The findings of this study have implications for the malaria control program. Transmission was occurring in the study area, and during 1987 there were 709 malaria cases and during the period January to June 1988, 585 cases. The inverse relationship between the percentage bloodfed and presence of DDT in a hut clearly indicates behavioral avoidance. This could result in an increase in biting of man outdoors. The replastering of the inner walls of huts by the inhabitants for social and practical reasons is a further impediment to control of An. arabiensis by house spraying with DDT; the percentage of bloodfed An. arabiensis caught leaving these huts and surviving was high. Surveys in the area subjected to the DDT vector control program showed that the percentage of huts replastered varied from 1.7 to 72% at different localities and times. Surveys in the study area found 25.7% of huts to be replastered in April/May 1981 and 46.6% in April/May 1982. The exit trap survival data indicate that from replastered huts there is a 20–24% greater survival of mosquitoes than from the DDT huts. Overall, this figure is expected to be in excess of 50% should the differential kill in replastered and DDT huts prior to capture in the exit trap be taken into account. A reduction in the replastering of huts should therefore result in more efficient vector control. K. Newberry (personal communication) found that replastering of huts was to a large extent carried out to combat biting by bed bugs and that their control by insecticidal spraying has led to a significant decrease in the replastering rate in the districts of Ubombo and Ingwavuma.

The data presented indicate that effective control of An. arabiensis will not be possible under the current control strategy. To increase the effect of vector control in the study area would require an integrated approach using more than just the annual application of DDT or preferably changing to an alternative insecticide with a reduced irritant effect on the vector species.

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