Transmission efficiency of *Culex quinquefasciatus* and *Aedes aegypti* to *Wuchereria bancrofti* infection: An experimental study*

Shailja Misra-Bhattacharya & K Tyagi

Division of Parasitology, Central Drug Research Institute, Lucknow 226001, India

Fax: 91-0522-223405; E-mail: root%@csedri.ren.nic.in

Received 8 February 2000; revised 21 June 2000

Lymphatic filariasis is a major tropical disease caused by mosquito borne nematode parasites. In India, two species of lymphatic dwelling human filariids, *Wuchereria bancrofti* and *Brugia malayi* are prevalent which are transmitted by two different species of mosquitoes viz. *Culex quinquefasciatus* and *Mansonia* spp. respectively. Due to high host specificity of these parasites most of the studies have been carried out using subperiodic strain of *B. malayi*. Nevertheless, attempts have also been made towards experimental transmission of periodic *W. bancrofti*. The vector used for experimental transmission of subperiodic strain of *B. malayi* has been McDonalds black eyed susceptible strain of *Aedes aegypti* whereas experimental transmission of *W. bancrofti* was made feasible by *C. quinquefasciatus*, *Anopheles gambiae* (natural vectors), *Aedes aegypti* and *A. togoi* (experimental vectors). So far, no concrete information is available on comparative transmission efficiency of natural and experimental vectors for experimental transmission of *W. bancrofti*. The present attempt has been made to evaluate the comparative susceptibility of *C. quinquefasciatus* and *A. aegypti* (McDonalds susceptible strain) to human bancroftian infection.

*Maintenance of vectors*—Mosquitoes were reared and bred in humidity (80±5%) and temperature (27±1°C) controlled insectarium and adults were fed daily on 10% glucose solution soaked in cotton.

*Microfilaraemic volunteers*—Few rural villages within the periphery of 40 km from Lucknow were identified as endemic for bancroftian filariasis with the help of medical and paramedical staff of primary health centre. Night blood smears (40 μl) of human subjects were examined for the presence of microfilariae (mf) using conventional night blood smear technique. For each donor, blood slides were made in duplicate. mf carriers (40) exhibiting variable mf counts (30-600 mf / 40 μl) were included in the present study.

*Infected mosquito feeding*—Mosquitoes (3-4 days old) were allowed to feed directly on human volunteer. For proper comparison on four occasions both the species of mosquitoes were allowed to feed simultaneously on different arms of the same volunteer. Feeding was carried out between 2100-2200 hrs in closed-door insectarium.

*Mf uptake by mosquitoes*—Number of mf ingested by individual mosquito was assessed by teasing at least 8 fully engorged mosquitoes from each cage on glass slide within half an hour of feeding. The smears were air-dried, dehaemoglobinized and stained with Leishman stain. The number of mf in each smear was recorded and average mf intake by a single mosquito of either species was calculated.

---

*Communication no. 5974
Assessment of L₃ recovery—After 12±1 days of feeding at least 10 mosquitoes from each cage were dissected and number of L₃ recovered from individual mosquito was recorded.

Statistical analysis—Mosquitoes of each species were fed on volunteers falling under different groups categorised on the basis of mf density in 40 µl blood. These were Group I (30-50 mf); Group II (51-100 mf); Group III (101-250 mf) and Group IV (400-600 mf). Data obtained from individually dissected mosquito fed on volunteers constituting one particular group were pooled for calculating the average number of mf ingested and L₃ recovered.

Pearson's coefficient of correlation was evaluated between (i) number of mf in donor's blood and average number of mf ingested (ii) number of mf in donor's blood and average number of L₃ recovered and (iii) average number of mf ingested and average number of L₃ recovered.

Complete development of L₃ from mf took place in both Culex and Aedes mosquitoes within 11 to 13 days post feeding at 27±1°C temperature and 80±5% RH. Observations on ingestion of mf by both the species of mosquitoes were made on 30 separate occasions using 4 groups of human volunteers as mentioned earlier. Frequency distribution of mean number of mf ingested and L₃ recovered per mosquito with respect to mf density in donor's blood (each group) is shown in Table 1. It is evident that mf intake by both the species of mosquitoes was directly related to mf density in donor's blood. The correlation was statistically significant in Culex mosquitoes (r = + 0.28). The number of mf ingested by Culex and Aedes was more or less similar when fed on blood carrying 30-250 mf / 40 µl. Aedes exhibited lower mf intake as compared to Culex when fed on heavily infected (400 mf / 40 µl) blood. Regarding L₃ recovery, considerable variation between the two species of vector was observed. In Culex the average recovery of L₃ was higher (range 0.81-4.04) than Aedes (range 0.86-1.95). However, the correlation between number of mf ingested and number of L₃ recovered was significant in Aedes (r=+0.81). The maximum recovery of L₃ from Culex occurred after feeding on blood containing 51-100 mf per 40 µl while Aedes required almost double the number of mf. The percent development of L₃ from mf in both Culex and Aedes has depicted in Fig. 1. It is obvious that ingestion of 4 mf by Aedes led to highest (22.5%) establishment of L₃ where as Culex demonstrated 48.5% L₃ establishment after ingestion of 11.5 mf.

C. quinquefasciatus is the most prevalent natural vector of W. bancrofti. These mosquitoes can easily be maintained in temperature and humidity controlled insectarium and have been used for experimental transmission of W. bancrofti from human to Indian langur, Presbytis entellus. As stated earlier another species of mosquito, A. aegypti which was made sus-

<table>
<thead>
<tr>
<th>Group of volunteer</th>
<th>mf / 40 µl blood</th>
<th>mf ingested Culex</th>
<th>L₃ recovered Aedes</th>
<th>mf ingested Culex</th>
<th>L₃ recovered Aedes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>30-50</td>
<td>3.89±0.78</td>
<td>0.81±0.23</td>
<td>2.75±0.42</td>
<td>0.86±0.16</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(50)</td>
<td>(40)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>II</td>
<td>51-100</td>
<td>9.5±0.63</td>
<td>4.04±0.31</td>
<td>10.3±0.80</td>
<td>1.14±0.36</td>
</tr>
<tr>
<td></td>
<td>(42)</td>
<td>(67)</td>
<td>(25)</td>
<td>(60)</td>
<td>(52)</td>
</tr>
<tr>
<td>III</td>
<td>101-250</td>
<td>9.6±0.72</td>
<td>2.10±0.21</td>
<td>9.95±0.69</td>
<td>1.95±0.05</td>
</tr>
<tr>
<td></td>
<td>(45)</td>
<td>(62)</td>
<td>(40)</td>
<td>(75)</td>
<td>(75)</td>
</tr>
<tr>
<td>IV</td>
<td>400-600</td>
<td>68.33±1.70</td>
<td>0.85±0.21</td>
<td>37.25±3.24</td>
<td>1.1±0.33</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(52)</td>
<td>(20)</td>
<td>(40)</td>
<td>(40)</td>
</tr>
</tbody>
</table>

Fig. 1—Percent development of W. bancrofti mf to L₃ in two species of mosquitoes fed on same donors simultaneously.
ceptible for subperiodic *B. malayi* has added advantage over *Culex*, in being a voracious feeder, does not prefer any biting time, takes very short time for blood feeding (personal observation) and its eggs can be preserved for months.

Although both *Culex* and *Aedes* have been shown to support the development of *W. bancrofti* mf to L3, remarkable differences in mf intake, L3 recovery and establishment of infection were obtained between these two vectors. It was interesting to observe that density of mf present in donor's blood at the time of feeding had direct correlation with number of mf ingested by individual mosquito. Other workers,5,6 also made similar observations. However, intake of mf was higher in *Culex* than *Aedes*, when fed on the same donor which could obviously be due to larger size of former5,1. It was interesting to note that both *Culex* and *Aedes* concentrated mf during feeding though this effect was more pronounced in the latter. Therefore, *Aedes* ingested almost comparable mf numbers as *Culex* inspite of its much smaller size. We have observed that *Culex* ingests almost three time more blood than *Aedes* (data not shown) thus expecting ingestion of mf three times more than *Aedes*. However, it was not so, as *Aedes* ingested either equal or half the number of mf ingested by *Culex*. It may thus be inferred that *Aedes* concentrated mf during ingestion around two to four times more than *Culex*. McGreevy et al.5,6 mentioned that both *Culex* and *Aedes* ingested more mf than expected with *Aedes* concentrating mf to higher degree. The recovery of L3 from both the species of mosquitoes varied considerably even after ingestion of similar number of mf. Better development of L3 was obtained from *Culex* as evidenced by higher L3 yield. The percent development of L3 in *Aedes* was maximum if 4 mf per mosquito were ingested. Nevertheless moderate recovery of L3 was obtained if ingested mf did not increase beyond 12. On the other hand *Culex* recorded 48.52% development on ingesting 11.5 mf/mosquito indicating that *Aedes* needs much less mf count in donor's blood than *Culex* for better L3 development. The wide variation in mf density in human blood does not affect the transmission potency of *Aedes* while in case of *Culex* mf counts in donor's blood is very crucial in determining the transmission potential. We did not take any donor with mf counts lower than 30 mf/40 µl, however McGreevy et al.5,6 have mentioned that low density carriers with 10 mf / ml in peripheral blood may serve as a potential mf carrier. In contrast to present findings regarding percent development of mf to L3 (between 2.85 and 22.5) in *Aedes* and (0.05 and 48.52) in *Culex*, the above authors claimed only 2.7 to 13% development of ingested mf into L3 in case of *Culex*. The present investigation, therefore, reveals that for experimental transmission of *W. bancrofti* to laboratory animals, *A. aegypti* may be preferred which has an edge over the natural vector *Culex* because of its easy laboratory maintenance, feeding behaviour and infection transmission pattern.

Sincere thanks are due to Mr R N Lai for the technical assistance. The co-operation of the Primary Health Centre, Kakori, Lucknow, in the fieldwork is gratefully acknowledged. Thanks are also due to DST, New Delhi for financial assistance and CSIR, New Delhi for the award of Senior Research Associateship to one of us (K.T.). CDRI communication No. is 5974.

References