COMPARATIVE SUSCEPTIBILITY TO WUCHERERIA BANCROFTI OF CULEX FATIGANS DELHI STRAIN AND OF STRAINS CYTOPLASMICALLY INCOMPATIBLE WITH IT

by

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ABSTRACT

The comparative susceptibility of Culex fatigans Delhi strain and of strains cytoplasmically incompatible with it was tested to Wuchereria bancrofti. Results of this study showed that all the strains which were tested were highly susceptible to infection and there was no consistent difference between the strains in their degree of susceptibility.

INTRODUCTION

Culex fatigans is the most important vector of bancroftian filariasis on the subcontinent of India. Due to rapid urbanization, C. fatigans and filaria are spreading to new areas, and presently the disease is endemic in almost all the States of India (Pandit, 1971). The WHO/ICMR Research Unit on Genetic Control of Mosquitoes is currently investigating the feasibility of eradication or control of C. fatigans by use of an integrated strain of C. fatigans, which is cytoplasmically incompatible with indigenous strains and carries a male-linked translocation as proposed by Laven & Aslamkhan (1970). This strain has cytoplasm of Paris origin and a male-linked translocation complex induced in chromosomes of the Delhi strain and has been backcrossed with a strain with Bangkok cytoplasm and Delhi genome, so as to combine the Paris cytoplasm with the indigenous genome, almost entirely of Delhi origin. The resulting strain has been designated as the IS-31B strain (Krishnamurthy & Laven, in preparation).

Since it is possible during genetic releases that any released strain might replace the local population and survive in the wild despite the disadvantage of a reduced fertility, it was considered advisable to compare the susceptibility of the indigenous and IS-31B strains to Wuchereria bancrofti before making releases in the field. It was recognized that differences would be unlikely because susceptibility would be under the control of chromosomal genes (Macdonald, 1967) and the backcrossing programme would ensure that the genome of females of the IS-31B strain was largely that of the Delhi strain.

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Before testing the susceptibility of the product of the five-time backcrossed IS-31B material, its immediate precursors, the products of the third and fourth backcrosses were also tested, as a strain designated D3, which contained Paris cytoplasm and Freetown genome and therefore a precursor of the IS-31B strain.

MATERIALS AND METHODS

This study was carried out at the National Institute of Communicable Diseases, Filaria Research and Training Centre, Varanasi (U.P.) and at WHO/ICMR Research Unit on Genetic Control of Mosquitos, New Delhi, India.

The following strains of C. fatigans were tested:

1. Delhi (Laboratory colony). This colony was started from approximately 5000 larvae collected at random from several villages in Delhi Union Territory in May 1970. It has been under continuous colonization on a mass scale and no fresh material from the field has been added.

2. D3 (Paris cytoplasm, Freetown genome).

3. Progeny of the third backcross of D3 to males of a strain with Bangkok cytoplasm and Delhi genome.

4. Progeny of fourth backcross of D3 to males of the strain described in No. 3 above. The males of this generation carried a male-linked translocation complex.

5. IS-31B strain: product of fifth backcross of D3 to males of a strain with Bangkok cytoplasm and Delhi genome and the males with a male-linked translocation complex.

All strains were reared and maintained under standard conditions to minimize the environmental effects on the vectorial capacity of different strains.

As W. bancrofti carriers are not available in and around Delhi, adult females of all were transported to Varanasi by air in specially designed insulated boxes for feeding on donors. Mortality during transportation among unfed and fed mosquitos was minimal and never exceeded 1%.

Female mosquitos five to six days old were fed on W. bancrofti infected subjects during the period of peak circulation in the peripheral blood, i.e. between 20.00 and 22.00 hours. Test and control groups were fed simultaneously on opposite hands of the same donor. Estimates of microfilaraemia were made from donors before and after exposure to feeding mosquitos and the mean value of microfilariae per cubic millilitre was determined.

After feeding mosquitos were transported back to the WHO/ICMR Unit laboratories and held at 28 ± 1°C and 80-85% relative humidity until dissections of the mosquitos were carried out on the tenth, eleventh and twelfth days after feeding. (The best results were obtained on the eleventh and twelfth day and if the dissections were done on the thirteenth day or later, there was a possibility of a few infective larvae escaping from mosquitos thus dissections were carried out on the eleventh and twelfth day.)

The index of experimental infection for different strains was calculated using the following modified formula of the WHO Expert Committee on Filariasis (1962):

For detailed histories of genetic strains see Krishnamurthy & Laven (in preparation).
\[ \text{a x d} \]

\[ \text{m} \]

\[ \text{a = Survival rate =} \frac{\text{No. of mosquitos surviving incubation period}}{\text{No. of mosquitos fed and surviving the return journey}} \]

\[ \text{d = Mean number of infective larvae per mosquito dissected =} \frac{\text{Total No. of infective larvae}}{\text{No. of mosquitos dissected}} \]

\[ \text{m = Mean number of microfilariae per cubic millilitre of donor blood at the time of feeding mosquitos.} \]

The statistical significance of the differences between the mean number of infective larvae per mosquito dissected (d) in the test (A) and control group (B) was calculated by the following formula (Dietz & Bekessy, personal communication):

\[ u = \left( \ln (d_A) - \ln (d_B) \right) \sqrt{\frac{N_A x N_B}{N_A + N_B}} \]

where

\[ d_A(d_B) \text{ is the mean number of infective larvae per mosquito dissected of strain A(B)} \]

and

\[ N_A(N_B) \text{ is the number of mosquitos dissected of strain A(B).} \]

Note: u must be greater than 1.96 for significance at 5% level.

The reason for using this formula is that the distribution of numbers of larvae per mosquito is extremely skewed, so that the "t" test cannot be used. The means were however found to approximate the standard deviations and the above transformation normalizes the distributions and makes the variances equal to 1.0.

RESULTS

Results of this study are given in Table 1. In the first series of experiments the D3 (with Freetown genome and without translocation) was compared with the Delhi strain. In one experiment the index of experimental infection and the proportion of mosquitos positive was higher in D3 and in the second both measures were slightly higher in the Delhi strain. The mean number of infective larvae per mosquito was not statistically significantly different in the two experiments. When progeny of the third backcross in preparation of the IS-31B strain was compared with the Delhi strain in the second series, no difference was found between the two groups in the index of experimental infection or proportion of mosquitos positive and there was also no difference observed in the mean number of infective larvae per mosquito dissected.

In the third series of experiments, out of the three comparisons a higher index of experimental infection was observed in the fourth backcross material in one comparison due to heavy mortality of the Delhi mosquitos whereas a significantly higher mean number of infective larvae per mosquito dissected was observed in the Delhi strain in two comparisons.

In the final series of experiments, when the IS-31B was compared with the Delhi strain, one comparison showed an index of experimental infection of IS-31B somewhat higher and a significantly higher mean number of infective larvae per mosquito dissected. In the other comparisons however the differences were in the opposite direction but were non-significant.
Thus in the nine comparisons made, two cases showed an index of experimental infection of the D3 strains and the backcrossed material to be higher than in the Delhi strain, one case in which the index for the Delhi strain was higher than for the backcross material and in the remaining six tests the indices were more or less the same as in the basic Delhi stock. The significance tests of differences between strains in mean number of larvae per mosquito dissected showed that, in six comparisons there was no significant difference, two comparisons showed a significantly higher mean in the Delhi strain, and one in the D3 backcross material. Considering only the fifth backcross (IS-31B strain), one comparison showed a significantly higher mean in the IS-31B material, and the other showed a non-significant difference in the opposite direction.

CONCLUSION

The results of this study showed that all strains tested were highly susceptible to infection and there was no conclusive evidence supporting differences in susceptibility between them. Therefore, if a wild population is replaced by the IS-31B strain, there appears no need for anxiety that this replacement would lead to a greater risk of infection by W. bancrofti due to change in vectorial capacity in the IS-31B strain.

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REFERENCES

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