EFFECTS OF *BRUGIA MALAYI* INFECTION ON THE SURVIVAL OF *ANOPHELES SINENSIS*

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Abstract. Effect of different numbers of infecting *Brugia malayi* on the survival of *Anopheles sinensis* were quantitatively studied in our laboratory. Four groups of healthy adult female mosquitos were tested. They were named G-0, G-1, G-2 and G-3, in which the numbers of microfilariae (mf) infecting per mosquito were 0, 5, 10 and 50, respectively. The experimental infection was conducted by inoculating the mf into the bodies of mosquitos through the neck membrane with a microinjector. It was observed that, in the groups from G-0 to G-3, the maximal life-span postinoculation (PI) were 21, 21, 20 and 10 days, the average life-spans PI were 7.78, 7.98, 7.05 and 3.55 days, and the survival time at 50% mortality PI were 6.72, 6.80, 6.40 and 4.00 days, respectively. Daily survival rates in the groups G-0, G-1 and G-2 declined slowly, over 25% on the 10th day PI, whereas the one in the group G-3 dropped down quickly, to zero on the same day. Linear regression analysis on the daily survival rates against the days PI showed significant differences between the groups G-0 and G-3 ($0.02 > p > 0.01$), but no significant differences between the groups G-0 and G-1, or between G-0 and G-2 ($p > 0.5$).

INTRODUCTION

The effect of filarial infection on the survival of arthropod vectors is an interesting topic in the host-parasite relationships, and investigation into this problem also has medical importance to the elucidation of the transmission dynamics of filariasis. Attention has been drawn to this field for a long period of time (Kershaw *et al*, 1953; Wharton, 1957; Lavoipierre, 1958; Townson, 1971; Christensen, 1978; Huang and He, 1988). However, previous studies in this context were all made by routine experimental infection, *ie,* the *per os* infection, either on the infected animals or through the artificial membrane of a blood-feeding device, and quantification of infecting filariae was derived from averaging the densities of microfilaremia and the quantities of blood taken by the vectors, or from counting the number of the third stage larvae (L3) recovered. Studies based on controlling the number of microfilariae (mf) infecting each vector host have not been reported. This is because it is very difficult or even impossible to do this employing routine methods.

Direct injection of pathogens into the bodies of arthropod hosts provides another way to the experimental infection (Nelson, 1962; Rosen and Gubler, 1974; Townson, 1975; Sucharit and Choochote, 1982). It gives a higher degree of quantitative precision (Cross *et al*, 1980; Jin and Xu, 1990). This paper reports a study using the technique of quantitative innoculation of *Brugia malayi* mf, the pathogen of malayan filariasis in Southeastern Asia, to the susceptible vector *Anopheles sinensis*, and observing the effects of different parasite burdens on the survival of these mosquitos.

MATERIALS AND METHODS

A laboratory strain of mosquito, *An. sinensis*, which was originally collected from the suburbs of Shanghai, was used in our study. They were reared, from eggs to adults, with routine methods in an insectarium under conditions of 28 ± 1°C and 80 ± 5% RH. Only healthy adult females less than 5 days old were sorted out for the experiment. They were allowed to engorge blood on restricted mice before infection. Periodic *B. malayi* mf were drawn from the peritoneal cavities of infective Mongolian jirds introduced from Guizhou Province, and suspended in physiological saline.

Experimental infection of mf to mosquitos was conducted by inoculation with an improved technique described earlier (Rosen and Gubler, 1974;

Xu *et al*, 1986; Jin and Xu, 1990). The whole set of inoculation apparatus was composed of a microinjector, a frozen pot, an operation plate and a stereomicroscope. The microinjector was kindly donated by Dr Gubler when he visited our laboratory several years ago. A glass needle, with a tip diameter in about 0.13 mm, was fixed on the operation rod of the microinjector.

Before inoculation, the mosquitos were randomly divided into four groups named G-0, G-1, G-2 and G-3, in which the numbers of mf inoculated per mosquito were 0, 5, 10 and 50, respectively. The G-0 group, *ie*, the control group, was injected with physiological saline with no mf. For the groups of G-1 and G-2, the dosages were determined by directly counting the mf by eye under the microscope. Owing to the difficulty in counting larger numbers of mf by eye, the dosage in the group G-3 was determined by sampling and averaging, that is, 10 samples were taken from the suspension and the numbers of mf inside were counted and averaged. If the average density of mf was beyond the requirement, physiological saline or mf was added to modulate the density.

Grouped mosquitos were then anesthetized by cooling in the -4°C freezing chamber of a refrigerator. Being immobile, they were transferred into the frozen pot which was composed of two different-sized glass cups, with the smaller cup being put inside the larger one and frozen water being placed between them. The inoculum, containing a definite number of mf in about 0.1 μl suspension, was injected through the neck membrane into the body of the mosquito by carefully operating the microinjector. The whole process was conducted under the stereomicroscope, and one sample of mf was used for inoculating one mosquito only. The experiment was performed from about 13 : 00 to 23 : 00.

After the inoculation, the mosquitos were put back into cages and reared in the insectarium under the conditions described above, fed with sugar and water. From then on, successive observations were carried out once a day until all the mosquitos died. The dead mosquitos were counted daily and cleaned out of the cages. When all observations were finished the indices which described the survival status of each group were calculated. Linear regression analysis on the daily survival rate against the days postinoculation was done. The results were compared between the control group (G-0) and each of the infected groups (G-1, G-2 and G-3).

RESULTS

The numbers of mosquitos in the groups of G-0, G-1, G-2 and G-3 were 205, 213, 215 and 220, respectively. About 15 minutes PI, most of the mosquitos revived from the chilly anesthetization and began to move and fly about. The mosquitos which lost the flight capability were construed as being dead. It appeared that the mechanical damage of the injecting operation on the survival of the mosquitos was not large, for the mortality in the control group (G-0) was reduced only about 5% in the first day PI. It was also showed that the wounds at the neck membranes of the mosquitos caused by the injection were well-repaired in a short time, leaving only a dark-colored spot at the point.

Four indices were used to describe the survival status of each group: the daily survival rate, the survival time at 50% mortality, the maximal life-span and the average life-span. The daily survival rate was derived from dividing the number of daily surviving mosquitos by the whole number of inoculated mosquitos in each group. The life-span at 50% mortality was the surviving time of a group when half of its members died. The maximal life-span was the surviving time of the members which lived the longest. The average life-span was derived from dividing by the whole number of mosquitos in a group by the sum which was obtained by adding the life-span of each mosquito together. When doing so, 0.5 day was subtracted from the number of the surviving days PI (*eg* when a mosquito died at the 5th day PI, its life-span was taken as 4.5 days). This is because the inoculations were conducted in the afternoon whereas the observations were carried out in the morning. The survival time at 50% mortality, the maximal life-span PI and the average life-span PI are illustrated in Table 1. It is obvious that for each of these indices the values were close to one another among the groups G-0, G-1 and G-2, but significantly reduced in group G-3.

The daily survival rates of each group were plotted against the days PI. The result showed that the daily survival rate in the group G-1 declined very slowly, being similar to the pattern of the group G-0. The daily survival rate in the group G-2 declined also steadily except for a dominant slope

Table 1

Comparison of some indexes showing the survival status PI between groups.

Groups	Dosages (mf/mosq)	No. mosq tested	Days PI at 50% mortality	Max life PI		Av life PI (days) (Mean ± SD)
				Days	No. mosq	
G-0	0	205	6.72	21	1	7.78 ± 4.44
G-1	5	213	6.80	21	2	7.98 ± 4.76
G-2	10	215	6.40	20	2	7.05 ± 5.25
G-3	50	220	4.00	10	3	3.55 ± 2.57

mf = microfilariae mosq = mosquitos No = Number of PI = Postinoculation
Max = Maximal Av = Average SD = Standard deviation

in the first day PI. The daily survival rates in these three groups, *ie*, G-0, G-1 and G-2 were all above 25% on the 10th day PI. However, the daily survival rate in the group G-3 dropped down more rapidly to zero on the 10th day PI, exhibiting two marked slopes on the first day and in the period from the 3rd to the 6th day PI (Fig 1).

In order to quantitatively examine the differences of the survival patterns between the groups, linear regression analysis on the daily survival rates against the days PI during the most significant period from the frist to the 7th day PI was made; this is the period in which *B. malayi* develops from mf to L3 in *An. sinensis* (Feng, 1936). Four linear regression equations were produced which expressed the changing tendency of the daily survival rates PI in these groups. It was shown that the regression line of group G-3 tilted more sharply, whereas they were almost the same among the

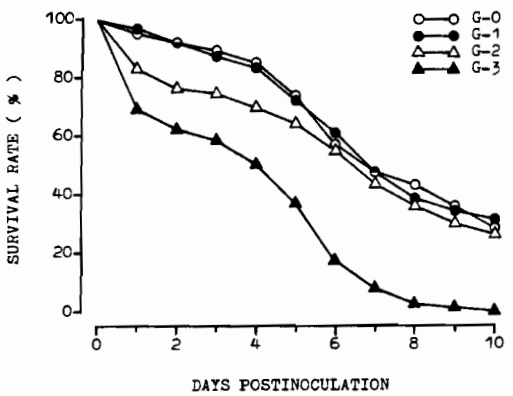

Fig 1–Daily survival rates of the four groups postinoculation (PI). The daily survival rates in the groups G-0, G-1 and G-2 declined slowly, being over 25% on the 10th day PI; whereas the one in G-3 dropped down quickly, landing zero on the same day. The slopes in the first day PI may be due to the mechanical damage caused by the injection and the disturbance of the active mf just inoculated. The slopes during 4-6th day PI may result from the rapid development of the filariae, which consumed large amount of nutrient substances of the mosquitos.

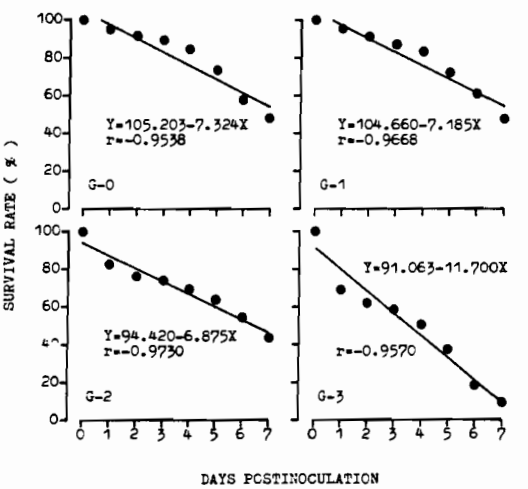

Fig 2–Linear regression analysis on the daily survival rates against the days postinoculation. The regression line of the group G-3 tilted more sharply, whereas those were almost the same among the groups G-0, G-1 and G-2. Student's *t* test on the comparisons of the regression coefficients showed significant difference between the groups G-0 and G-3 ($0.02 > p > 0.01$), whereas no significant differences between the groups G-0 and G-1, and between G-0 and G-2 either ($p > 0.5$).

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DISCUSSION

Some investigators have reported that mosquito vectors usually ingested more mf than expected, but others got contrary results (Wharton, 1960; Ramachandran, 1966; Kershaw *et al*, 1953; Obiamiwe, 1977). Several theories had been put forward to try to explain the variations (Harley, 1932; Gordon and Lumsden, 1939). However, this problem still exists now. In this situation, in order to obtain more precise quantification, we tried the substitutive way of experimental infection.

Inoculation had been in fact, used for a long time for the experimental infection of arthropod vectors with pathogens (Nelson, 1962; Rosen and Gubler, 1974; Sucharit and Choochote, 1982). Owing to the possibility of counting mf under the stereomicroscope, it seems more feasible for the quantitative study of filarial infection when the dosages are small. Studies on the development of filarial nematodes in mosquitos (both female and male) in order to genetically analyse the susceptibility of the vectors have been reported by several researchers (Cross *et al*, 1980; Townson, 1975). We ourselves have conducted the quantitative inoculation of *B. malayi* mf at dosages from 1 to 10 and observed filarial development in *An. sinensis* and *Culex quinquefasciatus* (Jin and Xu, 1990). However, the study of effects of filarial infection on the survival of mosquito hosts using the injection technique has not been reported. It may seem illogical in that the injecting operation itself will cause damage on the mosquitos. However, through our studies we demonstrated that this damage was not very great as long as the operation was careful. Additionally, we used the control group. This gave each group the same conditions and comparisons could then be made. As for the development of the inoculated filariae in mosquitos, it had been shown to be almost as normal as infecting *per os*, not only in morphology and the time taken, but also in their infection ability to the definite hosts (Terwedow, 1973; Xu *et al*, 1987).

Based on the more precise determination of the number of the infecting mf, our study showed that the filarial nematode *B. malayi* had only little detrimental effect on the life of mosquito *An. sinensis* provided the parasite burden was light. This may be due to the inherent adaptability of the relationships between the parasites and their vector hosts, which have been established during the long period of evolution and, therefore, guaranteed the transmission of the parasites. Similar findings had been reported by some researchers based on the routine *per os* infection (Wharton, 1957; Christensen, 1978; Ellrott, 1987). However, it should be noticed that a heavy parasite burden was intolerable to the mosquito hosts. This was clearly evidenced by the rapid reduction of the survival rate of *An. sinensis* when infecting *B. malayi* at a dosage of 50 in our study. From the data mentioned above we think that there may exist a threshold between the dosages of 10 and 50. When the dosage is below this threshold, the damage of filarial infection to the mosquito is too small to see; but, when the dosage is over it, the damage will grow dramatically. Further study, however, there is a need to find out this threshold definitely.

When analysing the curves of the daily survival rates of heavily infected mosquitos, we can see two dominant drops. The one occurred on the first day PI. This may be caused by the mechanical damage of the injection and by the disturbance of the active mf when they have just invaded the bodies of mosquitos. The other was around the 5th day PI. According to Feng (1936), the development of *B. malayi* from mf to L3 in *An. sinensis* took 6-6.5 days at about 30°C. So, we think the second drop of the survival curve may be mainly due to the rapid growth of the filarial larvae, which consume large amounts of nutrient substances in the mosquito hosts. The survival curve with some sharp dropping slopes, especially the one when the infecting parasites are approaching maturity, may be common characteristics of the relationships between the filarial nematodes and their mosquito hosts (Kershaw *et al*, 1953; Wharton, 1957; Townson, 1971; Ellrott, 1987). Mosquito hosts provide the filariae with suitable conditions for their growth and development. In return, this causes the damage of the hosts themselves. However, the damage become dominant, *ie*, the mortality increases rapidly, only when the parasite burden is beyond the threshold.

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