REPEATED INFECTIONS OF *BRUGIA PAHANGI* IN THE JIRD, *MERIONES UNGUICULATUS*

JOHN C. KOWALSKI* and LAWRENCE R. ASH

Division of Epidemiology, School of Public Health, University of California, Los Angeles, California 90024, U. S. A.

INTRODUCTION

Ash and Riley (1970 a, b) demonstrated the Mongolian jird, *Meriones unguiculatus*, to be a suitable host for the filarial worms *Brugia pahangi* and *B. malayi*. Studies to date using this *Brugia*-jird system have employed single inoculations of 5 to 75 infective-stage larvae into jirds (Ash, 1973). This study reports on the repeated infection of jirds with *B. pahangi* given at weekly or monthly intervals. It was our intention to determine what, if any, effect repeated inoculations have on the recovery and size of adult worms.

MATERIALS AND METHODS

The strains of *B. pahangi* and *Aedes aegypti* used, as well as the infection procedures have been described previously (Ash and Riley, 1970 a, b; Ash, 1973). Only male jirds were used in this study since they have been shown to be more susceptible to infection than females (Ash, 1971; Wesley, 1973). Multiply-infected jirds were inoculated alternately in the left and right groin. Weekly blood samples were collected from the jirds beginning 55 days post inoculation. The necropsy of jirds, recovery of adult worms, and fixation and measurement of adult worms were as described previously (Ash and Riley, 1970). Statistical analysis of the data was carried out using one-way analysis of variance ($P = 0.05$).

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* Present address: Department of Biological Sciences, State University College at Brockport, Brockport, New York 14420, U.S.A.

RESULTS

The protocol for infections and the number of adult *B. pahangi* recovered from the jirds are given in Table 1.

Single inoculation of infective larvae: Following a single inoculation of 75 infective larvae, 25% of the worms were recovered 30 days post inoculation. This yield decreased to 15.5% at 130 days post inoculation, however, little if any further decrease in yield was observed at 260 days post inoculation (14.8%). The female worms at 30 days post inoculation were approximately one-third the size of the 130 or 216 day-old female worms (8.31 ± 2.46 *vs.* 28.64 ± 5.00 or 31.28 ± 5.14 mm); there was no significant difference between the mean lengths of the 130 and 216 day-old female worms. The 30 day-old male worms were approximately three-fourths the size of the 130 or 216 day-old male worms ($8.88. \pm 1.40$ *vs.* 12.25 ± 1.80 or 12.96 ± 4.85 mm); there was no significant difference between the mean lengths of the 130 and 216 day-old male worms. No significant differences were observed in adult worm recovery or mean lengths of the male worms between group 2, receiving a single inoculation of 75 infective larvae, and group 3, receiving 4 weekly inoculations totaling 80 infective larvae. The female worms in group 3 were slightly but significantly shorter than the female worms in group 2 (26.61 ± 3.79 *vs.* 28.64 ± 5.00 mm).

There was no statistical difference in either the yield of adult worms or in mean worm lengths between groups receiving a single dose of 25 or 75 infective larvae.

Table 1

Protocol for infection of animals and the results obtained at necropsy.

Group[a] No.	No. of larvae × No. of doses	Total larvae	Age of worms (Days)	Average yield worms/jird	Average % yield worms/jird
1	75 × 1	75	30	18.75 ± 2.6	25.0
2	75 × 1	75	130	11.60 ± 3.1	15.5
3	20 × 4 (wk)[b]	80	113-134	12.80 ± 2.1	16.0
4	25 × 1	25	131	4.30 ± 0.9	15.2
5	75 × 4 (wk)	300	112-133	28.30 ± 9.4	9.4
6	75 × 1	75	216	11.10 ± 3.4	14.8
7	75 × 5 (mo)[c]	375	121-216	34.50 ± 10.5	9.5

[a] Grp. 6 = 10 jirds, all other groups = 6 jirds/grp; [b] Jirds infected once per week; [c] Jirds infected once per month.

Weekly Infections: The recovery of adult worms in group 5 which received 4 weekly doses of 75 infective larvae each, was 39% less than the % yield of adult worms in group 2, which received a single inoculation of 75 infective larvae (9.4% *vs.* 15.5%). There was no significant difference between the mean lengths of the male worms; however, the female worms in group 5 were significantly shorter than the female worms in group 2 (22.68 ± 4.50 *vs.* 28.64 ± 5.00).

Monthly Infections: The recovery of adult worms in group 7 which received 5 monthly doses of 75 infective larvae each, was 36% less than the yield of adult worms in group 6, which received a single inoculation of 75 infective larvae (9.5% *vs.* 14.8%). There was no significant difference between the mean lengths of the male worms, however, the female worms in group 7 were significantly shorter than the female worms in group 6 (27.22 ± 4.75 *vs.* 31.28 ± 5.14 mm).

Thirty Day-Old Infections: Several groups of jirds received an inoculation of 75 infective larvae 3 months after their last previous inoculation in order to assess the effects of older previous inoculations on a subsequent inoculation. The 3 month time span was chosen in hope that the two populations of worms could be separated based on their respective lengths. It was not possible to separate the different-aged populations of male worms although the female worms of the 2 groups could be separated. A mean of 10.0 thirty day-old female worms was recovered from jirds which had not received any larvae 3 months previously. A mean of 8.2 or 9.0 thirty day-old female worms were recovered from jirds which had received a prior inoculation of 25 or 75 infective larvae 90 days previously. A mean of only 4.5 thirty day-old female worms was recovered from jirds receiving previous monthly or weekly inoculations totaling 300 infective larvae. There were no statistical differences in the mean lengths of the 30 day-old females from previously uninfected jirds as compared to previously infected jirds.

Microfilaraemia Patterns: The mean prepatent period for all jirds used in the study was 67.4 days (range 58.5 to 76.5 days). There were no significant differences in the mean prepatent periods of the various groups of jirds; however, the microfilarial counts varied widely between jirds in the same group and between jirds in different groups. Microfilarial counts ranged from 0 to 200 micro-

filariae/20 c.mm blood during the course of the study. No differences were detected between the microfilaraemia patterns of the various infection groups.

DISCUSSION

The prepatent periods and number and size of worms resulting from single inoculations of 75 infective larvae compare favorably with the data reported by Ash and Riley (1970) and Ash (1973). There were no significant differences between the lengths of 4 and 7 month-old worms indicating that they reach their maximum size in the jird by 4 months post inoculation. According to Ash (1973), microfilarial levels do not rise substantially until after 6 months post inoculation. Since the jirds in this study were infected for only 1, 4 or 7 months it is difficult to draw any conclusions from the microfilaraemia patterns observed.

Based on our data it appears that as the number of inoculations and the number of larvae inoculated is increased, the number of larvae which become established decrease. The average yield of adult worms decreased from 15.5% following a single inoculation of 75 infective larvae to 9.4% in jirds receiving 4 weekly doses of 75 infective larvae each. This decrease in the establishment of new worms in multiply-infected jirds was also demonstrated by the recovery of 30 day-old female worms. The recovery dropped from a mean of 10.0 to 4.5 as the number of inoculations of 75 infective larvae was increased from 1 to 4. Since only a few dead or dying worms were recovered in any of the infection groups, it appears that any adverse effects of reinfection are directed mainly toward the ability of new incoming infective larvae to establish themselves in the jird. No differences in size or stage of development of the 30 day-old larvae were seen in the different infection groups indicating that once established the new incoming infective larvae can develop normally, at least for the first 30 days, despite the presence of the older adult worms. There did appear to be a modest stunting of the 130 and 210 day-old female worms in multiply-infected jirds, but no stunting of the growth of male worms. Denham *et al.*, (1972), working with *B. pahangi* in cats, also observed a decrease in the establishment of new worms as the number of inoculations was increased. They reported an 11% recovery of worms if 1 to 12 inoculations totaling 100 to 700 infective larvae were given; however, if 20 or more inoculations totaling more than 1,100 infective larvae were given the % yield dropped to 1 to 5%. Their data on worm length supports the conclusion that new larvae are prevented from becoming established in repeated infections.

It is apparent from the work of Macdonald and Scott (1953) that *Litomosoides carinii* in the cotton rat is much more sensitive to repeated infections than is *B. pahangi.* They observed a 40% reduction in the length of female worms introduced into a cotton rat which had received only one previous inoculation of 30 larvae as compared to female worms recovered from a previously uninfected rat. In addition, the larvae superimposed on a previous infection were late in completing their fourth molt and there was a decrease in the recovery of adult worms as the number of inoculations was increased.

The necessity of having a large number of living adult *B. pahangi* present in a host before any protective immunity is demonstrated against new incoming larvae partially explains the failure of previous workers to immunize animals against *B. malayi* using non-living antigens or X-irradiated infective larvae (Fredericks and Ramachandran, 1968; Ramachandran, 1970). It appears that in the case of *Brugia* spp. a very large stimulus is needed to provoke any protective immunity, undoubtedly a much greater stimulus than is induced by dead worm material or X-irra-

diated infective larvae. It is difficult to relate the results of this paper to any natural situation. Man and animals in endemic areas are most likely continually exposed to small numbers of infective larvae, rather than a few large doses of infective larvae. In order to more adequately approach a natural situation it will be necessary to infect jirds several times a week for many weeks with small doses of infective larvae.

SUMMARY

Male jirds, *Meriones unguiculatus*, were subcutaneously inoculated in the groin with 1 to 5 doses of infective-stage larvae of *Brugia pahangi* at weekly or monthly intervals. When a dose of either 25 or 75 larvae or 4 weekly doses of 25 larvae were given, 15-16% of the larvae were recovered as adults approximately 4 to 7 months post inoculation. Only 8-10% of the larvae were recovered if 4 weekly or 5 monthly doses of 75 larvae each were given. After an inoculation of 75 larvae, 25% of the worms were recovered at 30 days. The 30 day-old population consisted of an average of 10 female and 8.8 male worms. Jirds previously inoculated with 4 weekly or 5 monthly doses were challenged with an additional 75 larvae 30 days prior to necropsy. An average of only 4.5 thirty day-old female worms were recovered in these cases, presenting a 55% decrease as compared to the single inoculation situation. There was some decrease in the mean length of female worms in multiply-inoculated jirds, but no difference in the mean lengths of the male worm population from singly or multiply-inoculated jirds was observed. No differences in prepatent periods or in patterns of microfilaraemia were observed in singly or multiply-inoculated jirds.

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REFERENCES

ASH, L.R., (1971). Preferential susceptibility of male jirds *(Meriones unguiculatus)* to infection with *Brugia pahangi*. *J. Parasit.*, *57* : 777.

ASH, L.R., (1973). Chronic *Brugia pahangi* and *Brugia malayi* infections in *Meriones unguiculatus*. *J. Parasit.*, *59*: 442.

ASH, L.R. and RILEY, J.M., (1970a). Development of *Brugia pahangi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. *J. Parasit.*, *56*: 962.

ASH, L.R. and RILEY, J.M., (1970b). Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. *J. Parasit.*, *57* : 969.

DENHAM, D.A., (1972). Studies with *Brugia pahangi*. II. The effects of repeated infection on parasite levels in cats. *Int. J. Parasit.*, *2* : 401.

FREDERICKS, H.J. and RAMACHANDRAN, C.P., (1968). Notes on immunization studies against filarial infections. *In*: Proceedings of Seminar on Filariasis and Immunology of Parasitic Infections. Singapore; p. 103.

MACDONALD, E.M. and SCOTT, J.A., (1953). Experiments on immunity in the cotton rat to the filarial worm, *Litomosoides carinii*. *Expt. Parasit.*, *2* : 174.

RAMACHANDRAN, C.P., (1970). Attempts to immunize domestic cats with X-irradiated infective larvae of subperiodic *Brugia malayi*. I. Parasitologic aspects. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *1* : 78.

WESLEY, I.V., (1973). Sex hormones and their influence on preferential susceptibility of the male jird *(Meriones unguiculatus)* to infection by *Brugia pahangi* (Nematoda:Filarioidea). Doctoral thesis, University of California, Los Angeles.