

**The comparative susceptibility of male and female  
and of mature and immature cats to infection with  
sub-periodic *Brugia malayi***

by

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**Abstract:** An attempt was made to provide definitive information on the relative susceptibility of male and female and of immature and mature cats to experimental infection with sub-periodic *Brugia malayi*. Data from blood smears and from necropsies of cats used for various experimental procedures over a period of several years were reviewed. It was concluded that while immature cats were probably more susceptible to infection than mature animals, cats of any age can be infected with sufficient reliability to be useful in experimental studies. On the basis of larval worm recoveries and the presence of microfilaria in peripheral blood smears, there did not seem to be a demonstrable difference in susceptibility between male and female cats.

A review of the available literature shows that there is considerable variation among reports comparing the infection rates of males and females in areas where either *Wuchereria bancrofti* or *Brugia malayi* is endemic. These reports are complicated by variations in the habits and life styles, and thus the exposure of people to vector mosquitoes in different geographical areas. In general, the microfilaria rate in both males and females gradually rises with age until the peak is reached in early adult life. Because of the problem in determining the exact time and degree of exposure, it has been difficult to conclude what effect either age or sex has on the susceptibility of man to mosquito-transmitted filarial nematodes which inhabit the lymphatics.

Experimental animal models currently being used may permit a new look at the role which sex and age has on susceptibility to filarial infection. However, it is important to recognize that the influence which sex or age of the host has on susceptibility may vary under different experimental conditions and with different host-parasite combinations. In order to supply additional information on this subject, I have examined a number of cats which either served as infected but untreated controls for various experiments during the past several years or were

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observed for prolonged periods of time before they were subjected to any experimental procedures.

### MATERIAL AND METHODS

All cats used for larval or adult worm counts were infected on one hind foot with third stage larvae recovered from *Aedes togoi* mosquitoes fed approximately 2 weeks earlier on one of several cats with a moderate level of sub-periodic *Brugia malayi*. At designated times after inoculation, the animals were killed and examined for the number and condition of developing and adult worms. Infection and necropsy techniques have been described in earlier publications (Ewert and El Bihari, 1971; Ewert, 1971). In short, infective larvae administered to the hind foot of a cat generally accumulate in the afferent lymphatic vessels or in the subcapsular sinuses of the popliteal node of that leg. Larvae or developing worms seldom by-pass the popliteal node and migrate to the contralateral leg or other body sites. Thus, the number of worms recovered from these sites at necropsy represents a reliable measure of the total number of worms actually present. Most of the cats monitored for the presence of microfilaria were infected as indicated above but the inoculum varied. A few cats included in this group were infected by subcutaneous inoculation of larvae. In all cats which were kept longer than 10 weeks after inoculation with *B. malayi*, three 20 mm<sup>3</sup> peripheral blood smears were examined every 2 weeks. Microfilaremiæ were expressed as the number of microfilariae (mf) per 20 mm<sup>3</sup>.

For purposes of analysis, cats were grouped according to sex and age. Since most of the cats were of unknown background it was not possible to give an exact age. Cats in excess of 2000 g were considered mature because the smallest females, which had obviously littered, weighed more than 2000 g. Most of the cats listed as "immature" were actually between 800-1400 g and estimated to be from 2-1/2 to 4 months of age. Statistical analyses were based on the Student's "t" test with a p value of <0.05 considered as significant.

### RESULTS

In a series of experiments, 40 untreated control cats were killed 2 weeks after a single inoculation with 50 *B. malayi* larvae on one hind foot. By this time larvae had molted once, thus were in the 4th larval stage and were located in either the subcapsular sinuses of the popliteal lymph nodes or in the afferent lymphatic vessels. The mean number of larvae recovered from the 12 males was 27.2 (SE 2.3) with a range of 18-41. The mean number of larvae recovered from the 28 females was 25.0 (SE 1.7) with a range of 9-42. This difference between males and females was not significant.

When these 40 cats were grouped according to age, the mean number of larvae recovered from the 17 immature cats was 31.2 (SE 1.8) with a range of 19-42. The mean number of larvae recovered from 23 mature cats was 21.6 (SE 1.5) with a range of 9-33. This difference between the immature and mature cats, including both males and females, was significant. The mean number of larvae recovered from 6 immature males was 29.8 with a range of 19-41 while the mean number recovered from the 6 mature males was 24.5 with a range of 18-33. Because of the small sample size it is not possible to determine if this is a significant difference. The mean number of larvae recovered from 11 immature females was

31.9 (SE 2.1) with a range of 22-42 while the mean larval recovery of 17 mature females was 20.5 (SE 1.8) with a range of 9-30. This variation between immature and mature female cats was statistically significant.

A total of 59 female cats were monitored for the presence of mf for a period of 4 months or longer prior to treatment with any drug, reinfection with *B. malayi*, infection with a secondary microorganism or injection with contrast media for lymphography. Of these, 54 (92%) were positive for microfilaria at least once when three 20 mm<sup>3</sup> blood smears were examined every other week during the period of observation. Under these same conditions, 36 of 41 (88%) males were microfilaria positive. In animals kept for 9 months or longer, the peak mf level was frequently reached by 5 months after infection. However, in several instances the number of microfilariae per 20 m<sup>3</sup> continued to increase for more than a year after a single infection. There did not appear to be any consistent difference in mf levels, duration or time when the peak level was reached when male and female cats were compared or when comparing animals which had been immature when infected with those infected as mature animals.

## DISCUSSION

Ash (1971) cited several papers which suggested that the incidence of subperiodic *Wuchereria bancrofti* in the South Pacific, when measured by microfilarial surveys, was lower in females than in males once puberty had been reached and that females tended to have lower microfilarial densities than did males. Ash also reviewed literature dealing with the comparative susceptibility of males and females to experimental nematode infections including experimental *Litosomoides carinii* in cotton rats and surveys of dogs naturally infected with either *Dirofilaria immitis* or *Dipetalonema reconditum*. While there is evidence that in some nematode infections male animals are more susceptible than females, substantial experimental evidence for this being true in *Brugia* infections is limited to the jird.

Ash (1917) clearly showed that, based on both recovery of adult worms and the presence of peripheral microfilariae, male jirds (*Meriones unguiculatus*) were more susceptible to *Brugia pahangi* than females of corresponding age and background. This greater susceptibility to males to *B. pahangi* was confirmed by Ash (1973a) and extended to include *B. malayi* in jirds. Ash (1973b) also demonstrated that male jirds were more readily infected with *B. patei* than were females. El Bihari and Ewert (1973) confirmed that male jirds were more susceptible to *B. malayi* than were females of the same age and background. In each of these studies with jirds and *Brugia* spp., infective larvae were inoculated subcutaneously or, in a few instances, introduced via artificial puncture wounds.

When McCall *et al.* (1973) infected jirds with *B. pahangi* larvae by intraperitoneal inoculation, they recovered a high percentage of developing or adult worms in the peritoneal cavity of both males and females. Ah *et al.* (1974) found no difference between male and female jirds when examining them for either percentage of larvae recovered or distribution of larvae when the jirds had been infected by ocular inoculation. Gwadz and Chernin (1973) also showed a similar percentage of *B. pahangi* recovery from jirds of either sex when they were infected by the oral route. Thus, it appears that in the jird, where differential of susceptibility based on sex has been most clearly demonstrated, the increased susceptibility of the male may be influenced by either the route or site of inoculation.

By using the presence of circulating microfilariae as a criterion for infection, **Denham** (1974) showed that in sexually immature cats there was no significant difference between the susceptibility of males and females to *Brugia pahangi*. There was virtually no difference in the percentage of animals becoming infected and the prepatent period was essentially the same in both males and females. It did, however, appear that the mean number of microfilariae per mm<sup>3</sup> of blood was higher in males than in females when these determinations were made over a 10 week period starting 3 months after infection. Insufficient data were available to allow a meaningful comparison of the number of adult worms which developed in the two sexes.

Because of the variation in susceptibility of the female jird to infection with *Brugia* spp., depending on the route or site of inoculation, and the report of **Denham** (1974) showing little, if any, sex preference of *B. pahangi* in cats, I was prompted to examine data which had accumulated in our laboratory over a period of several years. From this data it seems certain that if the presence of healthy 4th stage larvae is considered a valid measure of susceptibility, then female cats can be infected as readily as males.

If the presence of circulating mf is used as a criterion of susceptibility, it again appears that there is no difference between the sexes since 92% of females and 88% of males were positive for mf. These animals were used for different experimental procedures; therefore, they were infected with different numbers of infective larvae and in some instances, after a period of observation, were reinfected with *B. malayi*, treated with drugs or infected with microorganisms. For this reason it was not possible to make a valid comparative analysis of mf levels over a uniformly prolonged period of time.

From a review of our experience involving a large number of cats infected with sub-periodic *B. malayi*, it appears that under these experimental conditions there is no evidence of a substantial, consistent difference in susceptibility between males and females. These observations confirm the work of **Denham** (1974) showing that the marked difference between male and female susceptibility to *Brugia* spp. reported earlier in jirds is not evident in cats. It would appear, therefore, that caution is needed in using experimental laboratory results to explain the findings of field surveys such as those of **Mataika et al.** (1971), **Joseph** (1971), and **Guptavanij et al.** (1971) in which males are more frequently identified as being infected with filaria. On the basis of early 4th stage larvae present shortly after infection, it would appear that immature cats of either sex were somewhat more susceptible to *B. malayi* than were mature animals. Unfortunately, although a number of cats were maintained for prolonged periods of time so that adult worms could be recovered, the number of animals in which the size of the inoculum and duration of the infection were similar was insufficient to permit a valid statistical analysis. Hopefully, additional observations under various experimental conditions and with different host-parasite combinations will help to understand the effects of age and sex on filarial infection and development.

While data presented in this paper have not served to clearly define what role either sex or age of the host has on susceptibility to *B. malayi*, it is reassuring to note that there is good evidence that either male or female and immature or mature cats can profitably be utilized in experimental studies of mosquito-transmitted, lymphatic-dwelling filariae.

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## RESUMEN

Se llevó a cabo un estudio para tratar de obtener informes concluyentes sobre la susceptibilidad de gatos, hembras y machos y entre jóvenes y adultos, a la infección experimental de *Brugia malayi* sub-periódica. También se revisó los datos de frotis sanguíneos y las necropsias de gatos que habían sido usados en experimentos durante varios años. Se llegó a la conclusión, que mientras los gatos jóvenes eran aparentemente más susceptibles a la infección que los adultos, se puede infectar a los de cualquier edad con la suficiente confiabilidad para que sean usados en estudios experimentales. Con base en la recuperación de larvas de *B. malayi* y en la presencia de microfilarias en los frotis de sangre periférica, no parece haber una diferencia significativa de susceptibilidad a la infección entre las hembras y los machos.

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