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# Experimental Infection of Rat with *Brugia pahangi*

Makoto SAKAMOTO, Yoshiki AOKI and Masaaki SHIMADA

*Department of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan*

Yasuo NAKAJIMA

*Department of Parasitology, Yamanashi Medical College Yamanashi, Japan*

**Abstract**: Susceptibility of rat (Wister strain) to *Brugia pahangi* infection was studied. Infective larvae, 100 to 300 in number, were inoculated subcutaneously in the inguinal region of 23 rats. Patent microfilaremia was found in 5 male and 1 female rats out of 20 (i. e. 9 males and 11 females) survived longer than 100 days after the inoculation. Male rat was more susceptible to *B. pahangi* infection than female rat. Mean prepatent period was 73 days. The microfilarial density of 5 rats with patent infection has never risen above 8 microfilariae per 60 cmm of blood, but one rat showed moderate level of microfilaremia exceeding 100 microfilariae per 60cmm of blood. Microfilarial periodicity in rats was not evident. Examination of microfilariae in the lungs was made on 10 rats of 14 which remained non-patent over 120 days postinoculation. All of 14 rats were also examined for adult worms. Two rats were positive for microfilariae in the lungs; from these rats no adult worm was recovered. Another 2 rats yieled one adult female worm each. Examination of the lungs to detect microfilariae seems to be indispensable for determination of patent infection in rats, because the procedure outlives a failure of adult worm recovery.

## INTRODUCTION

*Brugia pahangi* is a lymphatic-dwelling filarial worm of wild animals, and it has been experimentally transmitted to some laboratory animals. The jird and cat show high susceptibility to *B. pahangi* infection and seem to be adequate hosts. The rat, cotton rat and hamster are less susceptible and called inadequate hosts. The guinea-pig and mouse are grouped as resistant hosts (Sucharit and Macdonald, 1972).

Fox and Schacher (1976) and Weller (1978) recently suggested the possible use-

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fulness of the inbred rat as an experimental laboratory host of *B. pahangi* for the immunological and pathological studies of filarial infections. Although some literatures deal with the susceptibility of rat for *B. pahangi*, the usefulness of the rat as an experimental host for *B. pahangi* has not been elucidated thoroughly. The present study was undertaken to evaluate precisely the susceptibility and usefulness of the rat for *B. pahangi* infection.

## MATERIALS AND METHODS

Commercially available white rats (Wister strain) were used in this study. *Aedes aegypti* (Liverpool strain) were fed on the anesthetized dog with *B. pahangi* microfilaremia. Larval recovery from mosquitoes and larval inoculation into animals were the same as those described by Ash and Riley (1970). Infective larvae, 100 to 300 in number, were inoculated subcutaneously into the inguinal region of 23 rats. The blood samples (60 cmm) were obtained from the orbital sinus and examined for microfilariae at weekly intervals beginning 50 days after inoculation. The animals which remained in non-patent infection longer than 120 days post-inoculation were killed and examined for worms according to the technique of Ash and Riley (1970). At the time of necropsy, the lungs were cut crosswise and the blood films made from the vertical section of the lungs were examined for microfilariae. The periodical appearance of microfilariae in the peripheral blood was examined on two rats with patent infection. The blood was taken from the tail of the rats every two hours for a period of 24 hours.

## RESULTS

Out of 23 rats given infective larvae, 3 died before the blood examination for microfilariae. These 3 rats were extruded from the present study. Microfilariae were detected in the blood taken from the orbital sinus in 6 rats of 20 (30%) which were examined for microfilariae over a period of 120 days postinoculation. Preferential susceptibility of male rat was disclosed in the present study. Out of 9 male rats, 5 developed

Table 1. Microfilaremia rates in rats experimentally infected with *B. pahangi*

| Sex of animal | No. of examined | microfilaremia | | Percent positive |
|---|---|---|---|---|
| | | positive | negative | |
| male | 9 | 5 | 4 | 55.6 |
| female | 11 | 1 | 10 | 9.1 |
| total | 20 | 6 | 14 | 30.0 |

a patent infection. On the other hand, only one rat out of 11 female became positive for microfilariae in the peripheral blood (Table 1). The difference in susceptibility between male and female rats was highly significant ($p=0.038$) in the Fisher's exact probability test.

Prepatent periods ranged from 64 to 85 days. The mean prepatent period was 73

Table 2. Prepatent periods and number of microfilariae in peripheral blood of rats which developed patent infection

| Animal No. | Sex | Prepatent Period (days) | Weeks after the first appearance of microfilariae | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0* | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 1 | M | 64 | 1** | 11 | 14 | 4 | 3 | 34 | 20 | | 117 | | 74 | | 58 | | 67 |
| 2 | M | 71 | 2 | 0 | 0 | 0 | 0 | 1 | | 0 | | 1 | | 0 | | 0 | |
| 4 | M | 85 | 1 | 1 | 2 | 1 | | 3 | | 4 | | 2 | | 4 | | 2 | |
| 8 | M | 64 | 1 | 3 | 8 | 2 | 2 | 1 | 2 | | | | | | | | |
| 17 | M | 69 | 2 | 2 | 7 | 0 | 1 | 4 | 1 | 4 | 5 | | | | | | |
| 23 | F | 83 | 3 | 3 | 2 | 3 | 1 | 0 | | | | | | | | | |

* day when microfilariae appeared in the peripheral blood for the first time after inoculation of larvae.

** number microfilariae in 60 cmm of blood.

Table 3. Results of dissection of rats remained in non-patent infection over 120 days after inoculation of *B. pahangi* larvae

| Animal No. | Sex | Days sacrificed | Microfilariae in lungs | Number, sex and location of worms recovered |
|---|---|---|---|---|
| 5 | F | 149 | N.D. | − |
| 6 | F | 151 | N.D. | − |
| 9 | M | 191 | +++ | − |
| 10 | M | 192 | − | − |
| 11 | F | 193 | − | − |
| 12 | F | 196 | − | − |
| 13 | F | 123 | N.D. | − |
| 14 | F | 121 | N.D. | − |
| 16 | M | 175 | ++ | − |
| 18 | M | 175 | − | − |
| 19 | F | 188 | − | 1 adult female, hilar lymph node |
| 20 | F | 186 | − | − |
| 21 | F | 190 | − | 1 adult female, peri-renal fatty tissue |
| 22 | F | 192 | − | − |

F: female. M: male. N. D.: not done.

+++: 50 microfilariae or more/smear.

++: 10−49 microfilariae/smear.

days postinoculation. Only one of animals which became patent infection developed a moderate level of microfilaremia exceeding 100 microfilariae per 60 cmm of blood. The microfilarial density of the remaining rats has never raised above 8 microfilariae per 60 cmm of blood during the observations (Table 2).

Fourteen rats that failed to develop a patent infection were killed and examined for adult worms. The worms were recovered from 2 rats. One rat had one female worm in the hilar lymph node and the other, one female worm in the perirenal fatty tissue. From the remaining 12 rats, no adult worm was recovered. Ten rats were examined for microfilariae in the lungs immediately after the sacrifice. Microfilariae were detected in blood films from the lungs of 2 rats. These two rats had been negative for microfilariae in blood taken from the orbital sinus and failed to yield any adult worm recoverable at necropsy (Table 3).

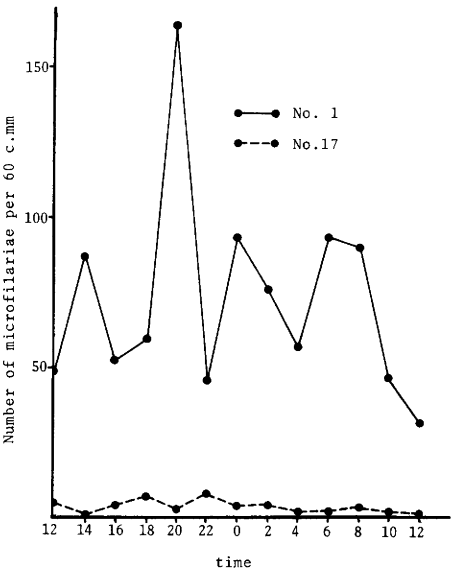


Fig.1. The 24-hour periodicity of microfilariae of *B. pahangi* in rat.

A study was made on two rats with patent infection to observe the microfilarial periodicity. Figure 1 shows the number of microfilariae present in blood samples taken every two hours for a 24 hours period. No apparent periodicity was detected in the peripheral blood of rats.

## DISCUSSION

The susceptibility of white rat probably differs much in the different strains of commercially available laboratory rats. The microfilarial positive rate of Carworth Farm strain was 48% (Harbut, 1973), of Delhi, 25% (Ahmed, 1967) and of Wister, 11.8% (Sucharit and Macdonal, 1972), respectively, We used Wister strain of rat in the present study. Our Wister rats were about 3 times as high in microfilariae positive rate as those of Sucharit and Macdonald (1972). The differential susceptibility to *B. pahangi* infection was also demonstrated in some inbred group of rats (Fox and Schacher, 1976).

The effect of sex and age of rat on the microfilarial rate has already been demonstrated by Sucharit and Macdonald (1972). Our study showed the preferential susceptibility of male rat to *B. pahangi* infection.

The mean prepatent period was reported to be 68 days in Carworth strain of rat (Harbut, 1973) and 153 days in Delhi rat (Ahmed, 1967). The prepatent period of our rat was varied from 64 to 85 days, with mean of 73 days. The prepatent period of Wister rat was close to those of Carworth strain.

The jird, an adequate host for *B. pahangi*, shows commonly high microfilaremia (Ash and Riley, 1970). The rat, however, shows usually low level of microfilaremia not exceeding 12 microfilariae per 20 cmm of blood (Harbut, 1973). Microfilarial density in our rats is generally as low as 1 to 8 microfilariae per 60 cmm of blood. The maintenance of *B. pahangi* in laboratory by the rat as an experimental host seems to be inadequate because of the low level of microfilaremia.

Ash and Riley (1970) and Malone and Thompson (1975) reported that the animals infected with *B. pahangi* often failed to develop the peripheral microfilaremia despite the presence of microfilariae in blood taken by heart puncture at necropsy. Shibata (1965) described that microfilariae of *Dirofilaria immitis* were most densely accumulated in the pulmonary capillaries. Their findings encouraged us to examine if microfilariae are localized in the lungs of rats which remain in non-patent infection. We could find microfilariae in the lung smears from 2 of 10 rats examined, though adult worm could not be recovered from these 2 rats. This fact suggested that examination of small volume of orbital blood is not always relied on to detect patent infection, and the examination of microfilariae in the lungs probably offers considerable advantages to determination of patent infection. Occasional failures of adult worm recovery have been reported in such experimental hosts as the rats, cotton rats and hamsters with patent *B. pahangi* microfilaremia (Ramachandran and Pacheco, 1965; Ahmed, 1967; Malone and Thompson, 1975). Our success in finding microfilariae in the lungs also indicates that the search for adult worms should be made more intensively and carefully.

The periodical appearance of *B. pahangi* microfilariae in the peripheral blood has been studied in naturally and experimentally infected animals. Semiperiodic appearance was reported in naturally infected cats and dogs (Edeson, 1959). On the other hand, no apparent periodicity was detected in the jird and cotton rat (Ash, 1973; Ramachandran and Pacheco, 1965). Sucharit (1973) observed two types of subperiodicity in Wister strain of rat. In the present study, however, no apparent periodicity was detected in Wister strain. Further studies into this problem seem to be necessary.

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*Brugia pahangi* のラットへの感染実験

坂本　信，青木克己，嶋田雅暁（長崎大学熱帯医学研究所寄生虫学部門）
中島康雄（山梨医科大学寄生虫学教室）

100〜300隻の *B. pahangi* 感染幼虫をラット（Wister 系）鼠径部皮下に接種し，*B. pahangi* に対するラットの感受性について検討した．実験ラット20匹（雄９，雌11）中６匹（雄５，雌１）の末梢血中に仔虫が検出されたが，残り14匹は接種後120日以上の観察で仔虫はみられなかった．雄ラットは雌に比し *B. pahangi* に対する感受性が明かに高い．感染幼虫接種より仔虫が末梢血中に出現するまでの期間（prepatent period）は平均73日であった．仔虫密度は一般に低く，６例中５例は60cmm 血液中仔虫数は８隻以下であった．ラットにおける仔虫の定期出現性はみられなかった．末梢血中に仔虫が認められないラット10例中２例では，肺臓内に仔虫がみとめられた．この２例とも成虫は全く回収されなかった．ラットを宿主として用いる実験においては，肺臓内の仔虫の検査は，patent infection の決定および成虫体の回収の失敗等を補う重要な意味をもつ．

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