

## Subcutaneous and Intraperitoneal Inoculation of *Dirofilaria immitis* Microfilariae into Mice

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### Introduction

Since the lack of a small laboratory host animal imposes limitations on research in human onchocerciasis, rodents have been used as proxy hosts (Nelson *et al.*, 1966; Rabalais, 1974; ElBihari and Hussein, 1980; Aoki *et al.*, 1980). Recently, Townson and Bianco (1982) reported on a promising model, *Onchocerca lienalis* microfilariae in CBA mice, that is a considerably promising one for immunological studies of onchocerciasis. As yet, the model of onchocercal microfilariae in rodents has not been fully utilized for further studies on onchocerciasis. A major reason is that most parasitologists face difficulty in obtaining large number of live microfilariae of *Onchocera* species. For this reason, it is of some urgency to find species of microfilariae which can survive in the skin of small hosts which are readily available in most laboratories. *Dirofilaria immitis* is a filarial worm of dogs which is widespread geographically and can be easily maintained in the laboratory. Although the microfilariae are usually found in the blood of dogs,

they do not have a sheath and they are morphologically similar to those of *O. volvulus*. Iwamoto (1972), Zielke (1980) and Ohga (1980) reported that *D. immitis* microfilariae survived in the blood of rodents for several weeks following their intravenous or intraperitoneal inoculation. The long life span of *D. immitis* microfilariae in rodents stimulated us to inoculate microfilariae subcutaneously into mice and to examine if the microfilariae would inhabit the skin of this host. The present paper describes the longevity and distribution of microfilariae of *D. immitis* in mice.

### Materials and Methods

Blood containing numerous microfilariae from a dog infected with *D. immitis* was mixed with 0.83% NH<sub>4</sub>Cl solution (1:6) to allow complete haemolysis. After repeated centrifugation at 300 g force for 10 min. at 5°C and washing with Hanks' solution several times, the number of microfilariae in a given volume was counted. A solution containing 10,000 microfilariae was inoculated into ICR mice subcutaneously in the inguinal region or intraperitoneally. Two mice, one male and one female were killed with ether at stated intervals. The ears and tail were removed, the mice were skinned, the eyes, heart, liver, spleen, kidneys, mesentery and genital organs were isolated. All tissues and organs were rinsed in Hanks' solution to remove adhering blood. The pelt and carcass were split into anterior and posterior portions at the level of the hypochondrium. To recover

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microfilariae, all tissues and organs were minced in Hanks' solution in petri dishes. After soaking for 2 hours at room temperature, the tissues were removed and the remaining fluid was centrifuged at 300 g force. The supernatant was discarded and sediment was examined for microfilariae. To recover microfilariae from peritoneal cavity, Hanks' solution was injected into the peritoneal cavity just before necropsy. After massaging the abdomen, washing fluid was collected from the peritoneal cavity and examined. To detect microfilariae in the blood, Knott's concentration technique (1939) was applied to 60 cmm of the blood taken from the orbital sinus by a fine capillary tube.

### Results

Table 1 represents the results of postmortem examinations of male and female mice inoculated subcutaneously with microfilariae. Active microfilariae were recovered from all animals necropsied until 4 weeks postinoculation. At 6 weeks, microfilariae were recovered from one of two animals. The recovery rates of microfilariae were 17.3 to 38.6 % of the original inoculum at 1 and 3 days following inoculation. At 5 days, the rates were 6.2 and 24.4 %. At 1 week, 6.5 % of inoculated microfilariae were recovered from one female mouse, whereas only 1.1 % were recovered from the other male. Only a few live microfilariae, 0.05 % of the original inoculum, were recovered from a mouse necropsied at 6 weeks. At 9 weeks and afterwards, microfilariae could not be found in any mice. Although microfilariae were detected in various parts of the body, the majority were found in the pelt and carcass. At 1 day after inoculation, microfilariae were accumulated in the posterior parts of the pelt and carcass. By 3 days, the microfilariae were evenly distributed in the pelt and carcass of whole body. A few microfilariae migrated to the tail, ears and eyes. Microfilariae were detected in orbital blood up to 1 week postinoculation. The microfilarial density was as low as 1 or 2 per 60 cmm of blood. The majority of microfilariae which migra-

ted to viscera were found in the heart and lungs. Only one microfilaria was recovered from the spleen throughout the observation period.

Table 2 gives the results of postmortem examinations of mice inoculated intraperitoneally. Active microfilariae were recovered from all mice necropsied until 4 weeks postinoculation. The recovery rates of microfilariae on 3 days and 5 days ranged from 29.8 to 34.6 % of the original inoculum. At 1 week, recovery rates dropped abruptly to less than 0.3 %. Microfilariae were detected in various parts of the body. However, the majority were recovered from the peritoneal cavity after it was rinsed with Hanks' solution. A few microfilariae were found in orbital blood at 5 days.

### Discussion

There are some reports on the introduction of *D. immitis* microfilariae into small laboratory animals. When microfilariae were given intravenously, they were detected for 4 weeks in the peripheral blood of rabbits (Iwamoto, 1972) and for 17 days in the blood of *Mastomys natalensis* and laboratory mice (Zielke, 1980). When microfilariae were inoculated intraperitoneally into mice, the great majority remained in the peritoneal cavity and only a few migrated to the blood (Ohga, 1980). In the present study, *D. immitis* microfilariae were inoculated into mice by subcutaneous and intraperitoneal routes. Live microfilariae were recovered mainly from the pelt and carcass over a period of 6 weeks following the subcutaneous inoculation. Approximately 27.9 % of the original inoculum were recovered at 24 hours, 3.8 % at 1 week, 0.6 % at 2 weeks and 0.1 % at 4 weeks postinoculation. So far as we know, the work presented here is the first to demonstrate that *D. immitis* microfilariae can survive in the skin of mouse for several weeks. The subcutaneous inoculation of *D. immitis* microfilariae into mice could be useful as a tool for the study of skin-dwelling microfilariae.

The longevity and distribution of *D. immitis* microfilariae in mice differ from those

Table 1 Distribution of *D. immitis* microfilariae in mice at various times after subcutaneous inoculation into the inguinal region

| Time of necropsy | Sex of mouse | % recovery | No. microfilariae recovered |      |       |       |         |       |         |      |               |        |     |      | Total |
|------------------|--------------|------------|-----------------------------|------|-------|-------|---------|-------|---------|------|---------------|--------|-----|------|-------|
|                  |              |            | Eyes                        | Ears | Pelt  |       | Carcass |       | Viscera | Tail | Orbital blood | Other* |     |      |       |
|                  |              |            |                             |      | lower | upper | lower   | upper |         |      |               |        |     |      |       |
| 24 hrs           | M            | 38.55      | 0                           | 1    | 1113  | 74    | 2530    | 32    | 20      | 0    | 0             | 0      | 85  | 3855 |       |
|                  | F            | 17.25      | 0                           | 1    | 862   | 69    | 661     | 65    | 41      | 1    | 1             | 1      | 24  | 1725 |       |
| 3 days           | M            | 26.44      | 0                           | 0    | 617   | 715   | 535     | 402   | 121     | 1    | 1             | 1      | 252 | 2644 |       |
|                  | F            | 18.09      | 0                           | 2    | 366   | 463   | 405     | 458   | 28      | 2    | 1             | 1      | 84  | 1809 |       |
| 5 days           | M            | 24.36      | 3                           | 13   | 367   | 624   | 441     | 469   | 138     | 2    | 2             | 2      | 377 | 2436 |       |
|                  | F            | 6.22       | 0                           | 0    | 91    | 169   | 91      | 158   | 86      | 1    | 1             | 1      | 25  | 622  |       |
| 1 W              | M            | 1.09       | 0                           | 0    | 9     | 28    | 13      | 31    | 10      | 0    | 1             | 1      | 17  | 109  |       |
|                  | F            | 6.51       | 1                           | 0    | 113   | 95    | 117     | 121   | 73      | 1    | 1             | 1      | 129 | 651  |       |
| 2 W              | M            | 0.33       | 0                           | 0    | 3     | 4     | 3       | 10    | 7       | 0    | 0             | 0      | 6   | 33   |       |
|                  | F            | 0.82       | 0                           | 0    | 10    | 4     | 15      | 6     | 26      | 0    | 0             | 0      | 21  | 82   |       |
| 3 W              | M            | 0.11       | 0                           | 0    | 1     | 0     | 1       | 2     | 2       | 0    | 0             | 0      | 5   | 11   |       |
|                  | F            | 0.92       | 0                           | 0    | 2     | 9     | 9       | 12    | 59      | 0    | 0             | 0      | 1   | 92   |       |
| 4 W              | M            | 0.04       | 0                           | 0    | 0     | 0     | 0       | 2     | 2       | 0    | 0             | 0      | 0   | 4    |       |
|                  | F            | 0.20       | 0                           | 0    | 1     | 0     | 7       | 1     | 3       | 0    | 0             | 0      | 8   | 20   |       |
| 6 W              | M            | 0.00       | 0                           | 0    | 0     | 0     | 0       | 0     | 0       | 0    | 0             | 0      | 0   | 0    |       |
|                  | F            | 0.05       | 0                           | 0    | 0     | 0     | 1       | 0     | 4       | 0    | 0             | 0      | 0   | 5    |       |
| 9 W              | M            | 0.00       | 0                           | 0    | 0     | 0     | 0       | 0     | 0       | 0    | 0             | 0      | 0   | 0    |       |
|                  | F            | 0.00       | 0                           | 0    | 0     | 0     | 0       | 0     | 0       | 0    | 0             | 0      | 0   | 0    |       |

\* Recovered from Hanks' solution rinsed tissues, organs, pleural and peritoneal cavities.

Table 2 Distribution of *D. immitis* microfilariae in mice at various times after intraperitoneal inoculation

| Time of necropsy | Sex of mouse | % Recovery | No. microfilariae recovered |      |      |      |         |         |      |               |        |      |  | Total |
|------------------|--------------|------------|-----------------------------|------|------|------|---------|---------|------|---------------|--------|------|--|-------|
|                  |              |            | Peritoneal cavity           | Eyes | Ears | Pelt | Carcass | Viscera | Tail | Orbital blood | Other* |      |  |       |
| 24 hrs           | M            | 79.14      | 7540                        | 0    | 6    | 176  | 109     | 19      | 0    | 0             | 64     | 7914 |  |       |
|                  | F            | 49.00      | 4620                        | 0    | 0    | 97   | 135     | 16      | 0    | 0             | 32     | 4900 |  |       |
| 3 days           | M            | 34.60      | 3026                        | 1    | 4    | 224  | 115     | 47      | 0    | 0             | 43     | 3460 |  |       |
|                  | F            | 32.14      | 2689                        | 0    | 0    | 37   | 85      | 196     | 0    | 0             | 207    | 3214 |  |       |
| 5 days           | M            | 30.26      | 2376                        | 1    | 1    | 64   | 231     | 93      | 2    | 3             | 255    | 3026 |  |       |
|                  | F            | 29.81      | 2949                        | 0    | 0    | 1    | 3       | 6       | 0    | 0             | 22     | 2981 |  |       |
| 1 W              | M            | 0.27       | 2                           | 0    | 0    | 3    | 8       | 5       | 2    | 0             | 7      | 27   |  |       |
|                  | F            | 0.10       | 0                           | 0    | 0    | 6    | 3       | 1       | 0    | 0             | 0      | 10   |  |       |
| 2 W              | M            | 0.08       | 2                           | 0    | 0    | 4    | 0       | 2       | 0    | 0             | 0      | 8    |  |       |
|                  | F            | 0.02       | 0                           | 0    | 0    | 0    | 2       | 0       | 0    | 0             | 0      | 2    |  |       |
| 3 W              | M            | 0.06       | 0                           | 0    | 0    | 0    | 2       | 2       | 0    | 0             | 2      | 6    |  |       |
|                  | F            | 0.04       | 0                           | 0    | 0    | 1    | 3       | 0       | 0    | 0             | 0      | 4    |  |       |
| 4 W              | M            | 0.02       | 0                           | 0    | 0    | 0    | 1       | 1       | 0    | 0             | 0      | 2    |  |       |
|                  | F            | 0.01       | 0                           | 0    | 0    | 0    | 0       | 1       | 0    | 0             | 0      | 1    |  |       |

\* Recovered from Hanks' solution rinsed tissues, organs and pleural cavity.

of microfilariae of *Onchocerca* spp. in mice. Our experiment with *D. immitis* microfilariae was lower in terms of longevity and recovery rates than the observation on microfilariae of *O. volvulus* (Aoki *et al.*, 1980) and *O. lienalis* (Townson and Bianco, 1982) in mice. When the subcutaneous route of infection was used, the microfilariae of *D. immitis* were evenly distributed in the pelt and carcass of whole body. The preferred sites of microfilariae of *Onchocerca* spp. are the ears and nose for *O. gutturosa* (Nelson *et al.*, 1966), the tail for *O. volvulus* (Aoki *et al.*, 1980), and the ears, nose and neck near to the site of inoculation for *O. lienalis* (Townson and Bianco, 1982). When the microfilariae of *D. immitis* were inoculated into mice intraperitoneally, the great majority remained in the peritoneal cavity. On the other hand, intraperitoneal inoculation caused microfilariae of *Onchocerca* spp. to migrate to ears and tail (Beveridge *et al.*, 1980; Aoki *et al.*, 1980; Townson and Bianco, 1982). *D. immitis* microfilariae invaded the blood early after infection, but *O. volvulus* microfilariae were not detected in orbital blood (Aoki *et al.*, 1980).

Ohga (1980) reported that *D. immitis* microfilariae persisted for 2 month, when mice were inoculated with  $10^6$  microfilariae intraperitoneally. In the present study, microfilariae were found in orbital blood up to 1 week after subcutaneous or intraperitoneal inoculation. These differences are probably due to the number of microfilariae inoculated by different authors.

Mice were reported to be infected intravenously with microfilariae of *Brugia* species (Grove *et al.*, 1979; Zielke, 1980). In another experiment, we inoculated *B. pahangi* microfilariae subcutaneously into mice, but failed to recover any live microfilariae at 3 days postinoculation (unpublished data). Sheathed microfilariae do not seem to survive long in mice when the subcutaneous route of infection is used.

### Summary

Microfilariae of *Dirofilaria immitis* were

inoculated subcutaneously into the inguinal region of mice. Live microfilariae were recovered up to 6 weeks postinoculation. During the early period of infection, the majority were recovered from the pelt and carcass near to the injection site. Later, microfilariae were distributed evenly in the pelt and carcass of entire body. Microfilariae invaded the viscera, blood, ears and tail, but they were few in number in those location. When an intraperitoneal inoculation was used, microfilariae were recovered exclusively from the peritoneal cavity and the recovery rate dropped abruptly at 1 week postinoculation.

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### References

- 1) Aoki, Y., Recinos, M. M. and Hashiguchi, Y. (1980): Life span and distribution of *Onchocerca volvulus* microfilariae in mice. *J. Parasitol.*, 66, 797-801.
- 2) Beveridge, I., Kummerow, E. and Wilkinson, P. (1980): Experimental infection of laboratory rodents and calves with microfilariae of *Onchocerca gibsoni*. *Tropenmed. Parasitol.*, 31, 82-86.
- 3) ElBihari, S. and Hussein, H. S. (1975): Location of the microfilariae of *Onchocerca armillata*. *J. Parasitol.*, 61, 656.
- 4) Grove, D. I., Davis, R. S. and Warren, K. S. (1979): *Brugia malayi* microfilariae in mice: a model for the study of host response to microfilariae. *Parasitology*, 79, 303-316.
- 5) Iwamoto, I. (1972): Studies on survival time of microfilariae. *Trop. Med.*, 14, 124-137.
- 6) Knott, J. (1938): A method for making microfilarial surveys on day blood. *Trans. Roy. Soc. Trop. Med. Hyg.*, 33, 191-196.
- 7) Nelson, G. S., Amin, M. A. Blackie, E. J. and Robson, N. (1966): The maintenance of *Onchocerca gutturosa* microfilariae *in vitro* and *in vivo*. *Trans. Roy. Soc. Trop. Med. Hyg.*, 60, 17.
- 8) Ohga, T. (1980): Basic studies on the *Dirofilaria immitis* and the experimental infection with larval filariae in mice. *J. Osaka*

- Med. Cent., 29, 745-764.
- 9) Rabalais, F. C. (1974): Studies on *Onchocerca cervicalis* microfilariae in the jirds *Meriones unguiculatus*. J. Helminthol., 48, 125-128.
- 10) Townson, S. and Bianco, A. E. (1982): Experimental infection of mice with the microfilariae of *Onchocerca lienalis*. Parasitology, 85, 283-293.
- 11) Zielke, E. (1980): On the longevity and behaviour of microfilariae of *Wuchereria bancrofti*, *Brugia pahangi* and *Dirofilaria immitis* transfused to laboratory rodents. Trans. Roy. Soc. Trop. Med. Hyg., 74, 456-458.

### ***Dirofilaria immitis* ミクロフィラリアのマウス皮下および腹腔内への感染実験**

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*Dirofilaria immitis* 感染犬から得たミクロフィラリアを ICR マウスの皮下および腹腔内へ接種後その分布を経時的に観察した。ミクロフィラリアは皮下接種後6週まで回収された。接種後早い時期にはミクロフィラリアは接種部位近くの皮膚、筋肉より多く回収され、その後全身の皮膚、筋肉に分布していた。少数のミクロフィ

ラリアの存在が内臓、血液、耳、尾等にみられた。腹腔内接種では大部分のミクロフィラリアが腹腔から回収され、接種後1週以降の回収率は急激に低下した。*D. immitis* ミクロフィラリアーマウスの実験系はオンコセルカ症のモデルとしてその有用性が確認された。