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Development of *Brugia pahangi* in the Peritoneal Cavity of Jirds—A Serial Jird to Jird Passage with Artificial Feeding to Vectors

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Introduction

Brugia pahangi, whose normal hosts are cats and dogs (Buckley and Edeson, 1956; Laing *et al.,* 1960), was transmitted to the jird (*Meriones unguiculatus*) with subcutaneous inoculation of infective larvae by Ash and Riley (1970). When the larvae were inoculated intraperitoneally into the jird, adults and microfilariae were exclusively recovered from the peritoneal cavity (McCall *et al.,* 1973). It is generally accepted that the worms localized in the peritoneal cavity seem to follow an aberrant mode of development which is different from the one that the lymphatic worms do (Katamine, personal communication). However, the microfilariae which were thus produced and localized in the peritoneal cavity of jirds were proved to reach infective larvae in the vector mosquitoes (Chuang *et al.,* 1979). It was also found that the worms in the peritoneal cavity could cause the production of IgG antibodies in the jird as the lymphatic worms do (Tomisato *et al.,* 1983). Although the microfilariae from jird peritoneal cavity and their developing larvae at the early stage were somewhat different in the localization of acid phosphatase from those derived from dog peripheral blood, the differences were not observed between the two larvae on and after the third day in vectors (Kimura *et al.,* 1983).

The present study was designed to investigate the unanswered questions relevant to the development of *B. pahangi* in the peritoneal cavity of jirds; further development of infective larvae derived from the microfilariae which were produced in the peritoneal cavity—if they would have potential to develop into adults; if the jird would show sexual difference in susceptibility after the intraperitoneal inoculation. Serial passage of intraperitoneal worms has established four generations of adults producing microfilariae in the peritoneal cavity of jirds.

Materials and Methods

The mosquitoes used were *Aedes aegypti*

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(Liverpool strain) which had been maintained in our laboratory.

From 7 days to 90 days after the intraperitoneal inoculation with 200 infective larvae which developed in the mosquitoes after direct feeding on an infected dog, 1 to 4 jirds were sacrificed at appropriate intervals, dissected and examined for the localization of worms. The technique used for worm recovery was described elsewhere (Sakamoto *et al.*, 1982).

To establish the first generation of intraperitoneal worms, each of 12 male and 22 female jirds was inoculated with 200 infective larvae which developed in the mosquitoes after direct feeding on infected jirds with microfilaremia.

The artificial feeding technique used was essentially the same as that described in the preceding report (Chuang *et al.*, 1979). Microfilarial sediment was resuspended in cold Dulbecco's phosphate-buffered saline without calcium or magnesium (PBS) to yield 600 to 700 microfilariae per 0.03 ml. The phagostimulant used was adenosine 5′-triphosphate (disodium salt, P-L Biochemicals Inc.) dissolved at the final concentration of 10^{-3} M just prior to the feeding.

To establish the second generation of intraperitoneal worms, 10 male and 9 female jirds were each inoculated intraperitoneally with 88 to 250 infective larvae which developed in the mosquitoes after artificial feeding on microfilariae from the peritoneal cavity.

The microfilariae which were produced by the second generation of intrapertoneal worms were recovered and fed artificially to the vector mosquitoes. To establish the third generation, 7 male and 5 female jirds were each inoculated with 50 to 200 infective larvae which developed thus in the vector mosquitoes.

The vectors fed on the microfilariae which were produced by the third generation of intraperitoneal worms through the artificial feeding device. To establish the fourth generation, 4 male and 11 female jirds were each inoculated with 150 to 200 infective larvae from the same vectors.

On and after 45 days postinoculation of infective larvae, the peritoneal cavity was irrigated with 3 ml of PBS every 3 to 5 days to examine the production of microfilariae.

Results

Most of worms were localized in the peritoneal cavity of jirds after the intraperitoneal inoculation with infective larvae from the mosquitoes which fed directly on

Table 1 Distribution of *B. pahangi* in jirds 7 to 90 days after intraperitoneal inoculation of 200 infective larvae from mosquitoes directly fed on an infected dog

Days dissected	No. of jirds examined	Total worms recovered	No. of worms detected in						
			pelt & carcass	heart & lungs	fatty tissue	mesenterium	kidney	genital organs	peritoneal cavity (%)
7	2	92							92 (100)
9	2	89						1	88 (99)
11	2	53	3				7	1	42 (79)
20	2	109					1		108 (99)
30	2	61	1			1			59 (97)
45	4	158	6	7		1	2	8	134 (85)
60	4	145	3	2		4	1	20	116 (80)
90	1	11	1			1			9 (82)

the infected dog (Table 1). Less than 20% of worms were found in other parts than the peritoneal cavity. The extraperitoneal worms seemed to have no decided preference among organs and tissues, although a few jirds harbored more worms in the kidney or genital organs.

Development to adults and production of microfilariae were observed in all animals inoculated with 200 infective larvae from the vectors which fed directly on the infected jirds with microfilaremia. In male jirds, the interval between the inoculation and the first detection of microfilariae in the peritoneal cavity ranged from 54 to 66 days with the mean of 61.00. In female jirds, the prepatent period for the first generation of intraperitoneal worms ranged from 55 to 67 days with the mean of 61.36 (Table 2). The difference of prepatent period between the males and females was not statistically significant (t=0.314).

The second generation of intraperitoneal worms was successfully established in 18 jirds out of 19 (Table 3). The prepatent period ranged from 64 to 71 days with the mean of 67.00 in male jirds, and from 65 to 70 days with the mean of 66.63 in female jirds. The difference of prepatent period between the males and females was not statistically significant (t=0.287).

The mean prepatent period was 61.24 for the first generation and 66.83 for the second generation. The difference was statistically significant (t=4.758).

The third generation was established in 11 jirds out of 12 (Table 4). The prepatent period ranged from 71 to 75 days with the mean of 72.14 in male jirds, and 69 to 75 days with the mean of 70.50 in female jirds. The difference between the males and females was not statistically significant (t=1.278). The mean prepatent period for the third generation was 71.54, significantly longer than that for the second generation (t=4.947).

Table 2 Prepatent period (*i. e.* intervals between inoculation and the first detection of microfilariae in the peritoneal cavity) of the first generation of intraperitoneal *B. pahangi*. Each jird received inoculation of 200 larvae derived from vectors directly fed on infected jirds with microfilaremia

Sex of jirds	Prepatent period in days	No. of jirds
M	54	4
M	62	2
M	64	2
M	66	4
F	55	3
F	56	2
F	60	4
F	61	5
F	63	1
F	65	2
F	67	5

Table 3 Prepatent period of the second generation of intraperitoneal *B. pahangi* in jirds inoculated with infective larvae from vectors taken microfilariae from peritoneal cavity

Jird number	Sex of jirds	No. of larvae inoculated	Prepatent period in days
1	F	200	67
2	M	200	65
3	F	180	65
4	M	200	66
5	F	88	66
6	F	200	70
7	F	200	70
8	M	150	64
9	M	150	64
10	M	150	71
11	M	150	71
12	M	150	71
13	M	200	64
14*	F	100	—
15	M	250	65
16	M	200	69
17	F	200	65
18	F	200	65
19	F	200	65

* negative for microfilaria on 71 days and died afterwards.

Table 4 Prepatent period of the third generation of intraperitoneal *B. pahangi* in jirds inoculated with infective larvae from vectors taken microfilariae produced by the second generation

Jird number	Sex of jirds	No. of larvae inoculated	Prepatent period in days
1	M	200	75
2	F	200	75
3	M	200	72
4	M	150	72
5*	F	50	—
6	M	200	72
7	M	200	72
8	F	200	69
9	F	200	69
10	F	200	69
11	M	200	71
12	M	200	71

* negative for microfilaria during observation period.

Table 5 Prepatent period of the fourth generation of intraperitoneal *B. pahangi* in jirds inoculated with infective larvae from vectors taken microfilariae produced by the third generation

Sex of jirds	No. of larvae inoculated	Prepatent period in days	No. of jirds
M	200	70	1
M	150	83	1
M	200	83	1
M	200	85	1
F	200	77	3
F	200	83	6
F	180	85	1
F	200	88	1

The fourth generation was established in all of 15 jirds (Table 5). The prepatent period ranged between 70 and 85 days with the mean of 80.25 in male jirds, and between 77 and 88 days with the mean of 82.00 in female jirds. The sexual difference was not statistically significant ($t=0.659$). The mean prepatent period for the fourth generation was 81.53, significantly longer than that for the third generation ($t=6.864$).

Discussion

The intraperitoneal inoculation of infective larvae into jirds was introduced as a convenient procedure to harvest a number of filarial worms for the biochemical and immunological studies. However, the view is widely held that the worms localized in the peritoneal cavity seem to follow an aberrant mode of development which is different from the one that the lymphatic worms do (Katamine, personal communication). So far as we know, the present finding is the first to reveal that the microfilariae produced in the peritoneal cavity of jirds can develop into adult worms and produce microfilariae through generations.

It is generally accepted that males are more susceptible to *Wuchereria bancrofti* infection than females (Murray, 1948; Katamine *et al.*, 1952: Nelson *et al.*, 1962). It has been also reported that male animals are more frequently infected with canine filariasis than females (Wallenstein and Tibola, 1960; Gubler, 1966). When *B. pahangi* is inoculated subcutaneously into jirds, the prepatent period has been demonstrated to be shorter for males than for females (Ash, 1971). In the present study, however, the sexual difference of prepatent period has not been observed through four generations of intraperitoneal worms. Although the numbers of inoculated larvae are larger in our experiment, the prepatent period, which elongates gradually through generations, appears to be comparable to that for male hosts inoculated subcutaneously in the report by Ash (1971) who computed the prepatent period in a way different from ours. In the subcutaneous inoculation, however, the microfilariae produced in the lymphatic vessels might stay in the pulmonary and cardiac blood for a while before they are detected in the peripheral blood. Assuming that this was the case, the first produc-

tion of microfilariae by the lymphatic worms in male hosts must be earlier than that by the intraperitoneal worms. Male sexual hormones seem to exercise an accelerating effect on the maturation of worms (Wesley, 1973; Devereux and Ash, 1978). If we accept this view, some of the peculiar findings could be explained. Because the intraperitoneal worms could be less influenced by the male sexual hormones than the worms localized in the testes, the former would mature slowly as the lymphatic worms in female hosts, resulting in the insignificant differences of their prepatent period between male and female hosts.

No possible explanation can be offered for the gradual elongation of prepatent period in the intraperitoneal worms through generations. Another unanswered question concerns to what extent the prepatent period would elongate as the generation goes by. Further studies into this problem are necessary.

Summary

More than 80% of worms were found in the peritoneal cavity, when the infective larvae of *Brugia pahangi* which developed in *Aedes aegypti* (Liverpool strain) were intraperitoneally inoculated into the jird (*Meriones unguiculatus*). The microfilariae from jird peritoneal cavity have been proved to have potential to develop into adults and produce microfilariae. Intraperitoneal worms were established in jirds until the fourth generation with the inoculation of infective larvae which were recovered from the mosquitoes after the artificial feeding on intraperitoneally-produced microfilariae. The prepatent period of intraperitoneal worms was not significantly different between male and female hosts. The prepatent period elongated gradually as the generation went by.

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スナネズミ腹腔における *Brugia pahangi* の発育——媒介蚊人工摂取によるスナネズミでの継代感染

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スナネズミ (*Meriones unguiculatus*) の腹腔内に *Aedes aegypti* (Liverpool strain) で発育した *Brugia pahangi* の感染幼虫を接種すると，80%以上の虫体が腹腔に検出された．スナネズミ腹腔由来のミクロフイラリアは成虫に発育し，ミクロフイラリアを産出する能力を持つことが証明された．腹腔内で産出されたミクロフイラリアを，人工摂取法を用いて蚊に摂らせ，回収した感染幼虫を接種して，スナネズミで，第4代まで腹腔内虫体を継代した．腹腔内虫体の潜伏期には，宿主の雌雄間で有意差を認めなかった．潜伏期は代を継ぐごとに次第に延長した．