Studies on *Brugia pahangi* in Inbred Hamsters

2. The intratesticular inoculation of infective larvae into CBN hamsters and some other rodents

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Abstract: Inbred CBN hamsters, Mongolian jirds and ICR mice were infected with *B. pahangi* by intratesticular inoculation of infective larvae. The animals were sacrificed within 30–70 days postinoculation and developing stages of larvae were recovered. In CBN hamsters, the average recovery rate was as high as 74.3%, of which 63.5% were obtained from the inoculated testes. When the inoculated testis and its peritesticular tissues, which can be excised *en masse*, are combined, 90% of the recovery were from these two sites.

The results obtained from jirds and mice were not encouraging due to the escape of larvae from the testis or the poor recovery rate.

Key words: *Brugia pahangi*, Hamster, *Meriones unguiculatus*, Technique for recovering.

INTRODUCTION

The developing 3rd-stage larvae in a host animal have been used in various studies on the prophylaxis of filariasis. The developing larvae in rodent hosts can also be served as a convenient model in studying mechanisms of anti-filarial effects of a drug, or in screening new anti-filarial drugs.

A difficulty in using this model exists in the recovery of larvae which spread to various directions from the inoculated site. The intraperitoneal inoculation of infective

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larvae (McCall *et al.*, 1973) or the use of diffusion chamber (Gass *et al.*, 1979; Court, 1982) will be useful techniques to solve this problem but they are not always satisfactory. Kimura *et al.* (in press) described a new technique to infect inbred GN hamsters with *B. pahangi* infective larvae by intratesticular inoculation. The method succeeded in producing "*in situ*" infection of *B. pahangi* in the testes of hamsters, and resulted in very high rates of larval recovery.

In the present study, the intratesticular inoculation method was tested on inbred CBN hamsters, Mongolian jirds and mice in order to know whether the method is applicable to other rodents.

MATERIALS AND METHODS

Infective larvae of *B. pahangi* were obtained from *Aedes aegypti* (Liverpool strain) which had been fed on microfilariae positive Mongolian jirds (*Meriones unguiculatus*) 11 days previously, using a Baermann funnel containing Hanks' balanced salt solution (HBSS). The larvae were washed five times with sterilized HBSS, and then 30 larvae were inoculated using a 1 ml plastic syringe fitted with a 22-gauge needle directly into the left testis of each animal.

A total of 7 inbred CBN hamsters of about 15 months old, 10 Mongolian jirds of 10 months old and 10 ICR mice of about 20 weeks old were used in this series of experiments. The CBN hamster (*Mesocricetus auratus*) is a inbred strain developed originally at Nippon Institute for Biological Science.

After the inoculation of infective larvae, a hamster and two mice per day were sacrificed at day 10 and then every 10 days for 60–70 days. For Mongolian jirds, two animals per day were sacrificed at 3, 7, 11, 20 and 30 days postinoculation. In order to recover developing larvae, visceral organs and tissues were resected systematically into separate Petri dishes containing HBSS, dissected, teased and then kept for 2–3 hours at 26 C to release larvae. The number of larvae recovered was recorded according to the site of recovery.

RESULTS

The results of larval recovery obtained from CBN hamsters are shown in Table 1. The average recovery rate of developing larvae by 70 days was 74.3%, of which 63.5% were obtained from the inoculated testes, and another 27.6% from the peritesticular tissues of the inoculated side. The "peritesticular tissues" include the epididymis, ductus deferens and adipose tissues attaching to the testis.

The results obtained from Mongolian jirds are shown in Table 2. The average

recovery rate until 30 days postinoculation was 47.7%, of which 26.6% were from the inoculated testes. Each of the peritesticular tissues of the inoculated side and "adipose

Table 1. The distribution and the recovery rate of developing stages of larvae of *Brugia pahangi* in inbred CBN hamsters

Day necropsied	10	20	30	40	50	60	70	Total
Animal No.	1	2	3	4	5	6	7	
No. larvae inoculated	30	30	30	30	30	30	30	210
Testis (inoculated)	25	6	10	11	9	20	18	99(63.5)***
Peritest.*(inoculated side)	1	16	12	4	5	5	0	43(27.6)
Testis (non-inoculated)	0	0	0	0	1	2	0	3(1.9)
Peritest.*(non-inoculated side)	0	0	0	0	0	0	0	0(0.0)
Heart and Lungs	0	0	0	0	6	0	4	10(6.4)
Carcass and Pelt	0	0	0	0	0	0	0	0(0.0)
Adipose tissues**	0	0	0	0	0	0	0	0(0.0)
Other organs and tissues	0	1	0	0	0	0	0	1(0.6)
No. larvae recovered	26	23	22	15	21	27	22	156(100)
% Recovery	87	77	73	50	70	90	73	74.3

* Peritesticular tissues (epididymis, ductus deferens and adipose tissues attaching to the testis).
** Axillar, inguinal and perirenal adipose tissues.
*** ( ): Percentages to the total recovery.

Table 2. The distribution and the recovery rate of developing stages of larvae of *Brugia pahangi* in Mongolian jirds

Day necropsied	3		7		11		20		30		Total
Animal No.	1	2	3	4	5	6	7	8	9	10	
No. larvae inoculated	30	30	30	30	30	30	30	30	30	30	300
Testis (inoculated)	16	9	2	7	1	3	0	0	0	0	38(26.6)***
Peritest.* (inoculated side)	0	5	1	1	3	4	2	1	7	4	28(19.6)
Testis (non-inoculated)	0	0	0	1	0	0	0	0	0	0	1(0.7)
Peritest.*(non-inoculated side)	0	0	0	0	0	0	0	2	0	2	4(2.8)
Heart and Lungs	0	0	2	1	1	1	2	0	0	0	7(4.9)
Carcass and Pelt	0	0	0	0	2	5	6	7	2	3	25(17.5)
Adipose tissues**	2	2	2	0	1	0	13	2	0	6	28(19.6)
Other organs and tissues	0	0	1	0	2	0	0	2	4	3	12(8.4)
No. larvae recovered	18	16	8	10	10	13	23	14	13	18	143(100)
% Recovery	60	53	27	33	33	43	77	47	43	60	47.7

*,**,*** :vide notes in Table 1.

tissues", which include inguinal, axillar and perirenal adipose tissues, accounted for 19.6 % of the total recovery. In general, larvae were found to move gradually out of the inoculated testis to the attaching peritesticular tissues, carcass and other parts of the animal body. At 20 days and thereafter, no larvae were recovered from the inoculated testes.

Table 3 shows the results obtained from ICR mice. Only few larvae could be recovered from different parts of the body at days 10, 20 and 30, but none after 40 days.

Table 3. The distribution and the recovery rate of developing stages of larvae of *Brugia pahangi* in ICR mice

Day necropsied	10		20		30		40		60		Total
Animal No.	1	2	3	4	5	6	7	8	9	10	
No. larvae inoculated	30	30	30	30	30	30	30	30	30	30	300
Testis (inoculated)	0	1	1	0	1	0	0	0	0	0	3(20.0)***
Peritest.* (inoculated side)	0	0	0	0	0	1	0	0	0	0	1(6.7)
Testis (non-inoculated side)	0	0	0	0	1	0	0	0	0	0	1(6.7)
Peritest.*(non-inoculated side)	0	0	0	0	0	0	0	0	0	0	0(0.0)
Heart and Lungs	1	0	0	0	0	0	0	0	0	0	1(6.7)
Carcass and Pelt	1	0	0	2	0	0	0	0	0	0	3(20.0)
Adipose tissues**	4	0	1	1	0	0	0	0	0	0	6(40.0)
Other organs and tissues	0	0	0	0	0	0	0	0	0	0	0 (0.0)
No. larvae recovered	6	1	2	3	2	1	0	0	0	0	15(100)
% Recovery	20	3.3	6.7	10	6.7	3.3	0.0	0.0	0.0	0.0	5.0

*,**,*** : vide notes in Table 1.

DISCUSSION

The developing stages of larvae in animal hosts have been used in immunological and chemotherapeutical studies on the prophylaxis of filariasis (Duke, 1961, 1963; Storey and Al-Mukhtar, 1982; Mak and Lim, 1983). They could also be utilized for primary screening of new anti-filarial drugs. The advantages of using the 3rd- and 4th-stage larvae are its easiness in infecting host animals and readiness in starting drug treatment. A problem with this model is the time-consuming process of recovering larvae. When infective larvae of *B. pahangi* were inoculated subcutaneously into the inguinal region of Mongolian jird, they spread to various directions and were distributed in the heart and lungs, genital organs, carcass, adipose tissues, etc. at 7—30 days (Shigeno *et al.*, 1983). Thus, to recover as many worms as possible, the dissection of the whole body of a host animal is required; and even if this was done, the recovery rate was only

30−50% (Shigeno *et al.*, 1983) in a *B. pahangi*-Mongolian jird model, which is believed to be a most suitable combination of host and parasite.

To facilitate the recovery of developing larvae, the intraperitoneal inoculation of infective larvae (McCall *et al.*, 1973) or the use of diffusion chamber (Gass *et al.*, 1979; Court, 1982) has been employed. The former method is widely used for its technical ease and high recovery rate (30 to 50%). However, the latter method seems to be less popular due to the technical complexity and the high mortality of larvae which occurs about two weeks after the implantation of the chamber.

Kimura *et al.* (in press) reported that, when *B. pahangi* larvae were inoculated directly into the testis of inbred GN hamster, they remained there and developed normally, producing "*in situ*" infection. The recovery rate of larvae by 47 days was 86 % on the average, and 87% of the recovery were from the inoculated testes. By this method, the inoculated testes could be used for histopathological studies.

In the present study, three kinds of rodents, inbred CBN hamsters, Mongolian jirds and ICR mice were tested for the intratesticular inoculation. Among these, only CBN hamsters gave promising results as an experimental host of *B. pahangi* larvae. Compared with other models ever reported, the average recovery rate of 74.3% is satisfactorily high, and furthermore, when the inoculated testis and its peritesticulars tissues are combined, more than 90% of the recovered worms are from these two sites. As these organ and tissues can be excised easily *en masse*, the process of recovering larvae will be greatly simplified. Other strains of hamsters might probably be used for the intratesticular inoculation.

In jirds and ICR mice, the results of larval recovery were disappointing. In the former, larvae were found to move out of the inoculated testis in about 10 days. In the latter, which have been reported to be a poor host of *B. pahangi* (Sakamoto *et al.*, 1982), only few worms or 5.0% of the inoculum could be recovered.

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近交系ハムスターにおける *B. pahangi* の研究　2．CBN ハムスター及び数種のげっ歯類に対する感染幼虫の睾丸内接種

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Brugia pahangi 感染幼虫を睾丸内接種法を用いて近交系 CBN ハムスター，スナネズミ，ICR マウスに感染させ，その後30−70日の間に動物を剖検して発育期幼虫の回収を試みた．CBN ハムスターでは平均74.3%という高い回収率が得られた．そのうち63.5%は感染幼虫を接種された睾丸より得られたものである．接種された睾丸及びその周囲組織は一塊として容易に切除されるが，この二つの部位を合せると全回収虫体の90%が得られることになり，従来多大の労力を要した幼虫の回収作業が著しく簡略化される．
スナネズミの場合には，睾丸外への幼虫移動がすみやかであり，またマウスでは幼虫の回収率がきわめて低く睾丸内接種法による利点は無かった．

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