

## The Comparison of Recovery Rate and Distribution of *Brugia pahangi* in Mongolian jirds (*Meriones unguiculatus*) Kept at Different Levels of Ambient Temperature and Ultra-violet Irradiation

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**Abstract:** Two groups of Mongolian jirds were kept at the ambient temperatures of 26C and 11.7C (average) respectively for up to 53 days after infection with infective larvae of *Brugia pahangi*, and the recovery rate and distribution of developing and adult worms in the animals were compared. There was no difference in the rate of recovery, but significantly more number of adult worms were recovered in the lower carcass in jirds kept at 26C than in those kept at 11.7C. Another group of jirds was UV-irradiated at the daily dose of 2000  $\mu$ W. min/ cm<sup>2</sup> for up to 75 days after infection, and the recovery rate and distribution of *B. pahangi* were compared with a non-irradiated control group. No differences were found between the two groups.

*Key words:* Mongolian jird, *Brugia pahangi*, Temperature, Ultraviolet.

### INTRODUCTION

It has been known that clinical manifestations caused by *Wuchereria bancrofti* infection vary by different endemic regions in the world (WHO, 1984). For example, chyluria was reported to be one of the commonest clinical manifestations in the temperate area such as Japan, but a rare condition in tropical countries, where elephantiasis and hydrocele cases were often abundant (Sasa, 1976). The difference in the localization of adult worms in a host seems to be related to the variations of chronic clinical manifestations. The factors which may influence the distribution of parasites will be differences in parasite strains, degree of exposure to infective bites and/or biting habits of vector mosquitoes. In addition, undefined factors such as climate, life style of people, nutritional conditions, etc. may also be influencing the distribution. The present paper deals

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with the effect of temperature and ultra-violet (UV) radiation, to which host animals are exposed, on the recovery and distribution of developing larvae and adult worms of *Brugia pahangi* in Mongolian jird (*Meriones unguiculatus*).

## MATERIALS AND METHODS

In the first experiment, 24 jirds of 12 months old were infected subcutaneously in the inguinal region with 100 infective larvae of *B. pahangi*, which had been obtained from *Aedes aegypti* fed on a microfilariae positive jird 11 days previously. The animals were then divided into two groups, one being kept at 26°C (high-temperature group: HTG) and the other at 4.5–19.5°C with the average of 11.7°C (low-temperature group: LTG). No humidity control was made in this experiment, but the experiment was conducted in relatively dry months in winter (January and February). Four animals from each group were sacrificed at days 5–8, 16–19 and 50–53 postinoculation and the worms were recovered following Ash and Riley (1970).

In the second experiment, 24 jirds of 12 months old were similarly infected and grouped. One group (non-irradiated) was kept in a room where UV radiation measured by a UV meter, UVX equipped with a UVX-25 sensor (Ultra-violet Products, Inc.), was less than 0.5  $\mu\text{W}/\text{cm}^2$ , and the other group (irradiated) was shaved on the back and received daily UV radiation at the dosage of 2000  $\mu\text{W}\cdot\text{min}/\text{cm}^2$  in two doses of 100  $\mu\text{W}/\text{cm}^2$  for 10 minutes using a XX-100 system (Ultra-violet Products, Inc.) which emits short-wave UV with the wave length of 254 nm. The temperature could not be controlled and fluctuated between 25–30°C. Four animals from each group were autopsied as above at days 4–6, 21–22 and 74–75 postinoculation.

For statistical analysis, Wilcoxon rank-sum test was used.

## RESULTS

The effect of ambient temperature on the recovery rate and distribution of developing larvae and adult worms is shown in Table 1. The average recovery rates for the HTG and LTG were 47.2% and 52.0% respectively, and there was no difference between the groups. In the early stage of infection, the majority of larvae were recovered from the lower carcass and the adipose tissues (inguinal and pri-renal) in both of the HTG and LTG. Then the larvae migrated with time to various directions. By days 50–53 when worms had been adults, the testes and peritesticular tissues, which include the epididymis, ductus deferens and adipose tissues attaching to the testis, have become the major site of worm recovery. A statistically significant difference ( $p=0.05$ ) in the distribution of worms was found at days 50–53 in the lower carcass, where the recovery rates in the HTG and LTG were 16.6% and 4.7% respectively. No other difference was observed.

Table 1. Recovery of *Brugia pahangi* in Mongolian jird (*Meriones unguiculatus*) kept under high (26°C) and low (4.5–19.5°C) temperatures

Site of recovery	High-temperature group			Low-temperature group		
	No. worms recovered at days*			No. worms recovered at days*		
	5 – 8	16–19	50–53	5 – 8	16–19	50–53
Testis and peritesticular tissues	17( 9.3)	53(26.1)	93(51.4)	21(10.4)	41(19.4)	126(59.7)
Heart and lungs	18( 9.9)	36(17.7)	23(12.7)	16( 8.0)	60(28.4)	32(15.2)
Upper carcass	5( 2.7)	6( 3.0)	5( 2.8)	2( 1.0)	11( 5.2)	4( 1.9)
Lower carcass**	63(34.6)	32(15.8)	30(16.6)	45(22.4)	34(16.1)	10( 4.7)
Pelt	1( 0.5)	16( 7.9)	4( 2.2)	10( 5.0)	4( 1.9)	1( 0.5)
Tail	0( 0.0)	4( 2.0)	11( 6.1)	0( 0.0)	1( 0.5)	3( 1.4)
Liver, spleen, kidney and mesenterium	2( 1.1)	5( 2.5)	2( 1.1)	3( 1.5)	1( 0.5)	10( 4.7)
Inguinal adipose tissue	44(24.2)	18( 8.9)	3( 1.7)	65(32.3)	13( 6.2)	10( 4.7)
Other adipose tissues	27(14.8)	26(12.8)	2( 1.1)	34(16.9)	38(18.0)	8( 3.8)
Washing fluid of organs and tissues	5( 2.7)	7( 3.4)	8( 4.4)	5( 2.5)	8( 3.8)	7( 3.3)
Total No. worms recovered (% Recovery***)	182(100) 45.5	203(100) 50.8	181(100) 45.3	201(100) 50.3	211(100) 52.8	211(100) 52.8

\* Days after inoculation of infective larvae.

\*\* Includes the seminal vesicle and urinary bladder.

\*\*\* A total of 400 infective larvae were inoculated in each group of days.

Table 2. Recovery of *Brugia pahangi* in UV-irradiated and non-irradiated Mongolian jird (*Meriones unguiculatus*)

Site of recovery	Control group			UV-irradiated group		
	No. worms recovered at days*			No. worms recovered at days*		
	4 – 6	21–22	74–75	4 – 6	21–22	74–75
Testis and peritesticular tissues	3( 2.3)	31(19.9)	68(45.6)	5( 4.4)	33(21.0)	66(48.9)
Heart and lungs	3( 2.3)	12( 7.7)	13( 8.7)	6( 5.3)	23(14.6)	13( 9.6)
Upper carcass	1( 0.8)	7( 4.5)	3( 2.0)	0( 0.0)	7( 4.5)	3( 2.2)
Lower carcass**	51(39.2)	65(41.7)	39(26.2)	44(38.6)	58(36.9)	34(25.2)
Pelt	3( 2.3)	3( 1.9)	0( 0.0)	0( 0.0)	7( 4.5)	0( 0.0)
Tail	0( 0.0)	10( 6.4)	3( 2.0)	0( 0.0)	4( 2.5)	9( 6.7)
Liver, spleen, kidney and mesenterium	1( 0.8)	2( 1.3)	6( 4.0)	2( 1.8)	5( 3.2)	7( 5.2)
Inguinal adipose tissue	60(46.2)	14( 9.0)	14( 9.4)	52(45.6)	11( 7.0)	3( 2.2)
Other adipose tissues	8( 6.2)	9( 5.8)	1( 0.7)	5( 4.4)	8( 5.1)	0( 0.0)
Washing fluid of organs and tissues	0( 0.0)	3( 1.9)	2( 1.3)	0( 0.0)	1( 0.6)	0( 0.0)
Total No. worms recovered (% Recovery***)	130(100) 32.5	156(100) 39.0	149(100) 37.3	114(100) 28.5	157(100) 39.3	135(100) 33.8

\*, \*\*, \*\*\*: vide note (\*, \*\*, \*\*\*) in Table 1.

The rate of recovery and distribution of worms were compared between UV-irradiated (UVG) and non-irradiated (NON-UVG) groups (Table 2). The average recovery rates in the UVG and NON-UVG were 33.9% and 36.3% respectively, and there was no difference between the two groups. The migration pattern of worms was largely the same as described in the first experiment, and no clear difference in distribution was found between the UVG and NON-UVG.

## DISCUSSION

The effects of body temperature on microfilariae in animal hosts have been studied by several workers. The number of circulating microfilariae of *Loa loa* was found to increase when the body temperature of host was raised, and vice versa (Hawking et al., 1967). On the contrary, lowering of the body temperature caused a rise in the count of microfilariae of *Brugia malayi* in a rhesus monkey (Hawking and Gammage, 1968). At extremely low body temperature levels (20-30C), microfilarial count of *Dirofilaria immitis* was observed to reduce remarkably (Katamine et al., 1960). Furthermore, Eberhard and Rabalais (1976) showed that both of the hypo- and hyperthermic stress on microfilariae of *Dipetalonema viteae* in Mongolian jird resulted in increased number of microfilariae in the peripheral blood. With regard to skin-dwelling microfilariae, Rabalais (1974) studied the distribution of *Onchocerca cervicalis* microfilariae inoculated subcutaneously into Mongolian jird and showed that the majority of microfilariae were in the skin of inguinal region in males, and in the skin of tail in females. He attributed the distribution to the microfilarial migration along a temperature gradient to the coolest regions of the host body. Bull and Cockett (1972) related the fluctuations in number of microfilariae of *Onchocerca volvulus* in skin snips to external temperature and humidity.

Most of these works have been conducted with the purpose of clarifying the mechanisms of microfilarial periodicity or fluctuations of microfilarial count, and the duration of animal exposure to a given temperature, which was usually unphysiologically high or low, was limited mostly in a few hours, or less than a week.

The present study reported on the effect of long-term ambient temperature on developing and adult filarial worms in terms of the recovery rate and distribution. It is known that the ambient temperature range of 0-25C, during which the LTG was kept in the present study, causes no abnormal deviation in jird body temperature, which is normally 36.7-37.7C (Eberhard and Rabalais, 1976).

The study revealed that the HTG showed significantly higher recovery of adult worms in the lower carcass than in the LTG. As the NON-UVG was kept at 25-30C without any treatment, the effect of high temperature on the distribution of adult worms was largely comparable with the HTG. When the comparison was made, there was no statistical difference in the two groups. It is very difficult to explain the mechanism and significance of the different distribution of adult worms in the lower carcass. It might be

suggesting the possibility that long-lasting low temperature as experienced in winter in the temperate climate influences the distribution of filarial worms and, perhaps, eventually the clinical picture of filariasis.

A group of jirds was UV-irradiated daily at the dose of 2000  $\mu\text{W}$ . min/cm<sup>2</sup>. This is 40 times more than the threshold limit value for human being (Last et al., 1980). The irradiation did not produce any noticeable physical and behavioral changes in the animals.

There was no significant effect of the UV irradiation on the recovery and distribution of *B. pahangi*. Mongolian jird was reported to be highly resistant to X-ray irradiation (Chang et al., 1964), and they may be also resistant to UV which has similar biological effects to X-ray. The dark skin of jird will reduce the effect of UV irradiation. References to be discussed on these matters seem to be almost lacking.

It was noticed that the recovery rate of temperature-treated groups (average 49.6%) was much higher than UV-treated groups (average 35.1%). This may be caused by the use of different batches of infective larvae.

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異なる環境温度・紫外線照射下に飼育されたスナネズミ (*Meriones unguiculatus*) における,  
*Brugia pahangi* の回収率及び体内分布の比較

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2群のスナネズミを、それぞれ環境温度 26°C と 11.7°C (平均) で、*Brugia pahangi* 感染幼虫接種後53日目まで飼育し、発育期幼虫・成虫の回収率と宿主内分布を比較した。回収率に差は無かったが、26°C で飼育された群では、11.7°C の群に比し、下半身の筋肉 (Lower carcass) より有意に多数の成虫が回収された。別のスナネズミ群を、感染後より75日間にわたり、紫外線1日照射量 2000 $\mu$ W $\cdot$ min/cm<sup>2</sup> を照射し、*B. pahangi* の回収率と分布を調べ、非照射対照群と比較した。両群に差を認めなかった。

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