Wuchereria Kalimantani Infected *Presbytis Cristatus* (A Primate Model for Drug Trials in Lymphatic Filariasis)*

Purnomo,† Bintari Rukmono‡

Abstrak

W. kalimantani adalah sebuah spesies baru dari genus Wuchereria yang secara morfologi dan taxonomi sangat mirip dengan W. bancrofti. Spesies baru ini adalah anggota genus Wuchereria pertama yang bentuk dewasanya ditemukan pada lutung (P. cristatus) secara alamiah. Percobaan telah dilakukan untuk mengembangkan P. cristatus yang terinfeksi W. kalimantani sebagai model primata untuk penapisan tersier filarisida baru. Kondisi optimal dan makanan yang cocok telah ditentukan. Infeksi optimal dicapai lewat 6 kali inokulasi mingguan, 50 larva L3, dan periode prepatennya kurang lebih 8 bulan.

Abstract

W. kalimantani is a new species, which is morphologically and taxonomically related to W. bancrofti. This new species is the first member of the genus Wuchereria in which adults have been recovered from naturally infected monkey (P. cristatus). Experiments were done to establish the W. kalimantani infected P. cristatus as a primate model in the tertiary screening of new filaricides. The optimal condition and the fixed menus tolerated by the monkey was determined. Optimal infection was reached with six weekly inoculations of 50 L3 larvae. The prepatent period was about 8 months.

***Keywords** : filaricides, tertiary screening*

INTRODUCTION

Lymphatic filariasis, caused by tissue dwelling nematodes *W. bancrofti, B. malayi and B. timori* is still a public health problem in the tropical countries. About 90 million people are currently infected in 76 countries and the majority lives in India, China and Indonesia. *W. bancrofti* is the predominant parasite. The disease affects mostly poor people in the rural areas.

The only drug now widely used is diethyl carbamazine (DEC) that kills the adult worms as well as the larvae. However, it gives serious side effects by multidose regimens so as to hamper the patients compliance and hence it is not a good drug for rational mass control. Therefore, new filaricides as potent as DEC and giving less side effect are needed. In order to start clinical trials in man, the candidate filaricides has to be screened in several animal models. The last of these models must be the closest to the human host and the human parasite in biological terms, i.e. a primate model. Until 1980, the suitable primate model of lymphatic filariasis caused by the Wuchereria genus was not available. In 1980, a new Wuchereria species (*W. kalimantani)* in a wild monkey (lutung, *Presbytis cristatus* was found by one of the authors (Purnomo).[1] A full description of the morphological characteristics of adult and microfilarial stages was made. [1]

The aim of this preliminary study is to determine the optimal condition of the *P. cristatus* in captivity and to infect it with *W. Kalimantani*, in order to perform well as a primate model in the tertiary screening of new filaricides.

METHODS

The monkeys were quarantained in a big cage (10 x 5 m), with small trees inside; 20 to 30 monkeys were put together. After a few months the monkeys were trans-

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* *Presented at the Symposium "Contribution of non-human primate research for human health" 15th Intern Primatologist Congress, 3-8 August, Denpasar, Bali*

† *NAMRU-II, Jakarta Detachement*

‡ *Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*

ferred to the living quarters in the laboratory, where they lived in single cages. A vetenarian and three attendants were taken care of the food to be given and the cleaning of the cages. The monkeys received BCG and rabies vaccinations.

We did some experimentation on the menu for the monkeys using local fresh leaves, vegetables , fruits, eggs and vitamins. Two monkeys were kept as a source of *W. Kalimantani*. Experimentations were also done to determine the amount of the L3 larvae and the repetitive numbers of inoculation to yield a maximal and constant microfilariaemia, and a shorter prepatent period. To obtain the larvae, the monkey was anaesthetized and its arm or limb was exposed to *A. togoi mosquitoes*. The L3 larvae were harvested from the mosquitoes after 24 - 30 days. The larvae was inoculated intracutaneously to the monkeys and microfilarial counts were done weekly.

RESULTS

The menus tolerated by the monkeys were fixed menus, which varied from day to day. Varieties of fresh leaves, many sort of vegetables like beans, stringbeans, carrots, tomatoes, cucumber, sweet potatoes and corn, fruits as banana and papaya are the ingredients. Boiled eggs are given twice a week. Vitamin B- complex and liver injection are given weekly, two tablets of vitamin C and vitamin B12 each are given weekly. Antibiotics are given at the occurrence of diarrhea or respiratory infection.

We succeeded in infecting the monkeys with its natural parasite, *W. Kalimantani*. Inoculations of an average of 100 and 300 L3 larvae given 1-3 times did give satisfactory results. In our Indonesian monkeys 6 weekly inoculations of 50 L3 larvae gave the most satisfactory results in terms of constant microfilarial findings and a shorter prepatent period namely 8 months. So, the time between the capture of the monkey until it is ready for use in chemotherapeutic trials is at least 11 months (adaptation time and prepatent period)

DISCUSSION

Rapid screening of potential filaricides was only available when Ash and Riley (1970) showed that *Meriones unguiculatus* was a good experimental host of *B. pahangi*.[2] The method therefore is to use *B. pahangi* and *Acanthocheilonema viteae* in *M. unguiculatus* for the primary screening of potential filaricides.[3] Further screening of compounds which have shown filaricidal activities in the primary screening is then carried out in the cat or dog infected with *B. malayi* or *B. pahangi*. Potential filaricides which have passed through these initial screenings will then be tested in the non-human primate model of lymphatic filariasis before clinical trials in man are considered. In recent years it is known that leaf monkeys have been extensively studied to determine their suitability as host for the lymphatic filarial parasite. It was found that the subperiodic *B. malayi - P. cristatus* primate model responded to Suramin and DEC as expected. Thereafter this model was used for tertiary screening of potential filaricides in Malaysia.[4]

Most attempts to rear *W. bancrofti* in a variety of animals including mongolian jirds, hamsters and cats failed to obtain complete development of the worm.[2,5,6] Later, *W. bancrofti* infective stage larvae were introduced into Macacca monkeys and it was found that the larvae were capable of reaching sexual maturity and producing microfilariae, but the microfilarial densities were low and erratic, and periodicity analysis could not be determined.[7]

We had determined the optimal condition of *P. cristatus* in captivity, and succeeded in infecting it with its natural parasite, *W. kalimantani*. *W. kalimantani* is morphologically and taxonomically related to *W. bancrofti*, therefore we suggest the *W. kalimantani* infected *P. cristatus* to be used as an animal model for tertiary screening of candidate drugs in lymphatic filariasis in Indonesia. This new model is unique for the fact that *W. kalimantani* is a new Wuchereria species that is found naturally in wild *P. cristatus* in Kalimantan (Borneo). This is the second species of the genus Wuchereria described and the first species that has been found to infect primates naturally.

CONCLUSIONS

- We had determined the optimal condition of *P. cristatus* in captivity and the monkeys tolerated the fixed menus given.
- To infect the monkeys, 6 weekly inoculations of 50 L3 larvae gave the most satisfactory results in terms of constant microfilarial findings and a shorter prepatent period.
- The *W. kalimantani* infected *P. cristatus* can serve as a primate model for tertiary screening of candidate filaricides in Indonesia.

Acknowledgment

The studies received financial support from the UNDP/World Bank/WHO special Programme for Research and Training in Tropical Disease. We wish to thank the filariasis team of the Department of Para-

sitology, Faculty of Medicine, University of Indonesia, and Dr. Wardhani from the Ragunan Zoo for the fine cooperation in the studies.

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