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## E.L.I.S.A. Using a Species Specific Monoclonal Antibody to Detect Infective Larvae of *Brugia malayi* in Vector Mosquitoes.

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## EXECUTIVE SUMMARY

The purpose of this project was to determine whether it is possible to control transmission of the human filarial parasite *Brugia malayi*, which is effected by mosquitoes, by using chemotherapy alone, without resorting to mosquito control measures. To this end, the average number of infective larvae of *B. malayi* to which inhabitants from two villages in Central Sulawesi (Indonesia) are exposed every year, the Annual Transmission Potential (ATP), was determined before treating the members of one of the villages with the filaricide drug diethylcarbamazine (DEC). Inhabitants of the other village were treated initially with a placebo. By the end of the third year, all of the participants were treated with DEC. The expectation was that the ATP in the village where people were treated would diminish, hopefully to a negligible value. The potential social and economic impact of such a result would be enormous, since it is easier and less expensive to administer DEC chemotherapy in selected villages than it is to implement widespread mosquito control programs.

To aid in the determination of the key parameter to be quantified in this study, the ATP, investigators from the University of Indonesia had available an immunological assay supplied by Tulane University. This test is based on a monoclonal antibody which is able to distinguish between two species of filariae that live together in the study area. These are the animal parasites *Brugia pahangi* and *B. malayi*. Infective larvae from these two parasite species cannot be distinguished one from the other by their shape. Hence, to obtain a true value of the ATP for *B. malayi* the test is very useful.

The determination of the ATP was completed. In the village whose inhabitants were treated with DEC (Walatana) the ATP decreased from 3280 in 1991-1992 to 96 in 1993-1994, a decrease of 97%. In the village of Mantikole (inhabitants not treated), the ATP also decreased, but much less, from 1602 in 1991-1992, to 584 in 1993-1994, a decrease of 67%.

We are very pleased with the results. Although it was not possible to completely interrupt the transmission of *B. malayi* by chemotherapy alone, it was possible to diminish transmission very significantly. Hence, we hope that our approach will be seriously considered as an inexpensive option for the control of transmission of Malayan filariasis.

## RESEARCH OBJECTIVES

The overall aim of our project, as stated in the original grant application was to determine whether transmission of the human lymphatic filaria *Brugia malayi* can be terminated by application of diethylcarbamazine (DEC) chemotherapy alone, without vector control measures.

To achieve this aim, the following objectives were to be accomplished:

- **1. To perform a longitudinal determination, over a one year period prior to DEC treatment, of the Annual Transmission Potential (ATP) of *B. malayi* in two villages of Central Sulawesi, where both the animal parasite *Brugia pahangi* and the anthropophilic form of *B. malayi* are endemic.**

- **2. To perform a complete screening of the villagers for microfilaremia and adenolymphangitis followed by DEC treatment of the inhabitants of one village and placebo treatment of those of the other.**

- **3. To reassess the ATP of *B. malayi* as above over a two year period after DEC treatment.**

- **4. To reassess microfilaremia and adenolymphangitis rates of the villagers at the end of the study period. To treat the inhabitants of the village given placebo with DEC.**

**All of these objectives were accomplished.**

## METHODS AND RESULTS

### Accomplishment of objective (1)

### Determination of the Annual Transmission Potential for *B. malayi* in two villages of Central Sulawesi: Mantikole and Walatana.

A 10 month mosquito collection period in the village of Mantikole, and a 9 month collection period in Walatana were completed. Ten all night collections were regularly performed in each village. The mosquitoes were separately stored during each collecting hour, identified and counted by species the following morning, and dissected for filarial larvae. Results are as follows:

a) 143,761 mosquitoes were collected in Mantikole (Table 1) and 51,775 in Walatana (Table 2).

b) 8,017 *An. barbirostris* biting man indoors and outdoors were collected in Mantikole and 9,625 in Walatana, comprising 5.6% and 18.6% of the mosquito fauna (Table 3).

c) In Mantikole, the infection rate was 2.5% and the infective rate 1.3%; in Walatana 3.5% and 1.7%, respectively (Table 3).

d) Infection occurred indoors as well as outdoors in both villages (Tables 4 and 5).

d) Each infective mosquito harbored 4.4 L3s in Mantikole, and 5.0 in Walatana Tables 1, 2, and 3).

e) Each person received a total of 364 (101x3/10x12) infective bites per year in Mantikole and 652 (163x3/9x12) in Walatana (Data for this calculation in Tables 1 and 2; summary in Table 3).

f) **The Annual Transmission Potential, defined as the average number of infective larvae received per person each year was 1,602 in Mantikole and 3,280 in Walatana.**

**TABLE 1**

**LANDING COLLECTIONS OF MOSQUITO VECTORS IN MANTIKOLE AND THEIR INFECTION RATES**

| | OCT | NOV | DEC | JAN | FEB | MAR | APR |
|---|---|---|---|---|---|---|---|
| MOSQ. COLLECTED | 5679 | 11454 | 26930 | 19250 | 20639 | 14378 | 9380 |
| *An. barbirostris* | 142 | 338 | 995 | 1461 | 1351 | 592 | 231 |
| Infected *An. barbirostris* | 12 | 14 | 25 | 30 | 37 | 22 | 12 |
| Mos. with L1/No L1 | 0/0 | 5/8 | 9/25 | 11/30 | 11/43 | 5/13 | 2/5 |
| Mos. with L2/No L2 | 2/10 | 2/6 | 0/0 | 8/22 | 8/28 | 6/16 | 2/7 |
| Mos. with L3/No L3 | 10/47 | 7/29 | 16/63 | 11/46 | 18/92 | 11/53 | 1/1 |

| | MAY | JUN | JUL | TOTAL |
|---|---|---|---|---|
| MOSQ. COLLECTED | 8246 | 14972 | 12833 | 143761 |
| *An. barbirostris* | 255 | 850 | 1802 | 8017 |
| Infected *An. barbirostris* | 5 | 20 | 30 | 200 |
| Mos. with L1/No L1 | 2/2 | 5/17 | 15/65 | 65/208 |
| Mos. with L2/No L2 | 1/2 | 2/8 | 3/8 | 34/107 |
| Mos. with L3/No L3 (Infective *An. barbirostris*) | 2/18 | 13/62 | 12/34 | 101/445 |

Mosquito collection: 10 days each month for 10 months

## TABLE 2

### LANDING COLLECTIONS OF MOSQUITO VECTORS IN WALATANA AND THEIR INFECTION RATES

| | NOV | DEC | JAN | FEB | MAR | APR |
|---|---|---|---|---|---|---|
| MOSQ. COLLECTED | 5473 | 11813 | 9180 | 5334 | 5925 | 1884 |
| *An. barbirostris* | 387 | 1096 | 1624 | 1576 | 1877 | 669 |
| Infected *An. barbirostris* | 22 | 35 | 41 | 51 | 67 | 37 |
| Mos. with L1/No L1 | 7/33 | 15/44 | 11/49 | 17/46 | 21/69 | 10/31 |
| Mos. with L2/No L2 | 2/9 | 6/11 | 8/30 | 9/38 | 13/46 | 11/33 |
| Mos. with L3/No L3 | 13/49 | 14/32 | 22/114 | 25/139 | 33/173 | 16/51 |

| | MAY | JUN | JUL | TOTAL |
|---|---|---|---|---|
| MOSQ. COLLECTED | 3099 | 5210 | 3859 | 51774 |
| *An. barbirostris* | 899 | 853 | 644 | 9625 |
| Infected *An. barbirostris* | 36 | 24 | 23 | 336 |
| Mos. with L1/No L1 | 12/34 | 7/22 | 7/25 | 107/353 |
| Mos. with L2/No L2 | 7/12 | 3/7 | 7/61 | 66/252 |
| Mos. with L3/No L3 (Infective *An. barbirostris*) | 17/122 | 14/55 | 9/91 | 163/820 |

Mosquito collection: 10 days each month for 9 months

## TABLE 3

### COMPARISON OF *An. barbirostris* IN MANTIKOLE AND WALATANA IN RELATION TO THE FILARIAL ENDEMICITY OF THE TWO VILLAGES

| | MANTIKOLE | WALATANA |
|---|---|---|
| NO. *An. barbirostris*/TOTAL NO. OF MOSQUITOS COLLECTED | 5.6% | 18.6% |
| INFECTION RATE | 2.5% | 3.5% |
| INFECTIVE RATE | 1.3% | 1.7% |
| NO. OF L3/INFECTIVE MOSQUITO | 4.4 | 5.0 |
| INFECTIVE BITES/YEAR | 364 | 652 |
| NO. L3 A PERSON RECEIVED/YEAR | 1602 | 3280 |
| MICROFILARIA RATE | 16% | 21% |
| ACUTE CLINICAL FILARIASIS | 30% | 37% |

## TABLE 4

### INDOOR RESTING COLLECTIONS OF MOSQUITO VECTORS IN MANTIKOLE AND THEIR INFECTION RATES

| | OCT | NOV | DEC | JAN | FEB | MAR | APR |
|---|---|---|---|---|---|---|---|
| NO. MOSQ. COLLECTED | 319 | 783 | 942 | 797 | 1001 | 766 | 500 |
| *An. barbirostris* | 15 | 15 | 35 | 54 | 73 | 21 | 10 |
| Infected *An. barbirostris* | 0 | 2 | 1 | 1 | 0 | 2 | 0 |
| Mos. with L1/No L1 | 0/0 | 0/0 | 1/2 | 1/3 | 0/0 | 0/0 | 0/0 |
| Mos. with L2/No L2 | 0/0 | 1/1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| Mos. with L3/No L3 | 0/0 | 1/1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |

| | MAY | JUN | JUL | TOTAL |
|---|---|---|---|---|
| MOSQ. COLLECTED | 303 | 791 | 460 | 6662 |
| *An. barbirostris* | 7 | 37 | 48 | 315 |
| Infected *An. barbirostris* | 0 | 0 | 1 | 7 |
| Mos. with L1/No L1 | 0/0 | 0/0 | 0/0 | 2/5 |
| Mos. with L2/No L2 | 0/0 | 0/0 | 0/0 | 1/1 |
| Mos. with L3/No L3 | 0/0 | 0/0 | 1/2 | 4/8 |

## TABLE 5

### INDOOR RESTING COLLECTIONS OF MOSQUITO VECTORS IN WALATANA AND THEIR INFECTION RATES

| | NOV | DEC | JAN | FEB | MAR | APR |
|---|---|---|---|---|---|---|
| NO. MOSQ. COLLECTED | 83 | 247 | 496 | 209 | 311 | 51 |
| *An. barbirostris* | 7 | 23 | 135 | 126 | 147 | 20 |
| Infected *An. barbirostris* | 0 | 1 | 4 | 3 | 6 | 2 |
| Mos. with L1/No L1 | 0/0 | 1/3 | 1/4 | 1/3 | 1/4 | 0/0 |
| Mos. with L2/No L2 | 0/0 | 0/0 | 0/0 | 1/3 | 2/5 | 1/1 |
| Mos. with L3/No L3 | 0/0 | 0/0 | 3/8 | 1/7 | 3/11 | 1/9 |

| | MAY | JUN | JUL | TOTAL |
|---|---|---|---|---|
| MOSQ. COLLECTED | 94 | 141 | 74 | 1706 |
| *An. barbirostris* | 38 | 25 | 27 | 548 |
| Infected *An. barbirostris* | 2 | 0 | 1 | 19 |
| Mos. with L1/No L1 | 1/1 | 0/0 | 0/0 | 5/15 |
| Mos. with L2/No L2 | 0/0 | 0/0 | 0/0 | 4/9 |
| Mos. with L3/No L3 | 1/1 | 0/0 | 1/8 | 10/44 |

**Accomplishment of objective (2)**

**Complete screening of the villagers for microfilaremia and adenolymphangitis followed by DEC treatment of the inhabitants of one village and placebo treatment of those of the other.**

i) PARASITOLOGICAL SCREENING (MICROFILARAEMIA).

Night blood samples were collected from all of the villagers in both villages between 20.00-24.00 hours. The blood films were processed and microfilariae counted the following morning. The following results were obtained.

a) In both villages, the microfilaria (mf) rates were higher in males. These differences could not be explained by the biting behavior of the vector (Tables 6 and 7).

b) Microfilaremia started late in childhood (5-9 years) in both villages and reached a plateau level thereafter.

c) The mf prevalence in Mantikole was slightly lower than that in Walatana.

**TABLE 6**

**MICROFILARIA PREVALENCE RATE IN MANTIKOLE, CENTRAL SULAWESI, INDONESIA**

| | FEMALES | | | MALES | | | TOTAL | | |
|---|---|---|---|---|---|---|---|---|---|
| AGE | NO. | MF+ | (%) | NO. | MF+ | (%) | NO. | MF+ | (%) |
| 0-4 | 16 | 0 | 0 | 24 | 0 | 0 | 40 | 0 | 0 |
| 5-9 | 14 | 2 | 14 | 27 | 1 | 4 | 41 | 3 | 7 |
| 10-19 | 39 | 5 | 13 | 33 | 6 | 18 | 72 | 11 | 15 |
| 20-29 | 29 | 5 | 17 | 33 | 10 | 30 | 62 | 15 | 24 |
| 30-39 | 19 | 3 | 16 | 25 | 7 | 28 | 47 | 10 | 21 |
| 40-49 | 12 | 1 | 8 | 14 | 5 | 36 | 26 | 6 | 23 |
| 50-59 | 10 | 2 | 20 | 12 | 4 | 33 | 22 | 6 | 27 |
| >60 | 3 | 0 | 0 | 5 | 1 | 20 | 8 | 1 | 13 |
| | 142 | 17 | 12 | 173 | 34 | 20 | 315 | 51 | 16 |

**TABLE 7**

**MICROFILARIA PREVALENCE RATE IN WALATANA, CENTRAL SULAWESI, INDONESIA**

| | FEMALES | | | MALES | | | TOTAL | | |
|---|---|---|---|---|---|---|---|---|---|
| AGE | NO. | MF+ | (%) | NO. | MF+ | (%) | NO. | MF+ | (%) |
| 0-4 | 13 | 0 | 0 | 11 | 0 | 0 | 24 | 0 | 0 |
| 5-9 | 31 | 4 | 13 | 27 | 8 | 30 | 58 | 12 | 21 |
| 10-19 | 57 | 11 | 19 | 45 | 12 | 27 | 102 | 23 | 23 |
| 20-29 | 30 | 2 | 7 | 28 | 13 | 46 | 58 | 15 | 26 |
| 30-39 | 33 | 6 | 18 | 27 | 5 | 19 | 60 | 11 | 18 |
| 40-49 | 15 | 1 | 7 | 26 | 11 | 42 | 41 | 12 | 29 |
| 50-59 | 5 | 2 | 40 | 8 | 1 | 13 | 13 | 3 | 23 |
| >60 | 2 | 0 | 0 | 4 | 1 | 25 | 6 | 1 | 17 |
| | 186 | 26 | 14 | 176 | 51 | 29 | 362 | 77 | 21 |

Blood sample = 20 µl (thick blood smear)

ii) CLINICAL SCREENING (ADENOLYMPHANGITIS).

All villagers were examined for histories and clinical signs of adenolymphangitis and scar tissues (objective signs of past adenolymphangitis).

a) Prevalence of acute filariasis was slightly higher in males than in females in the village of Mantikole, and twice as high in the males of Walatana, compared to the females in this village (Tables 8 and 9). The overall prevalence was slightly higher in Walatana (37%) than that in Mantikole (30%).

b) There were only 6 persons with elephantiasis in these two villages.

**TABLE 8**

**CLINICAL PREVALENCE RATE IN MANTIKOLE, CENTRAL SULAWESI, INDONESIA**

| | FEMALES | | | MALES | | | TOTAL | | |
|---|---|---|---|---|---|---|---|---|---|
| AGE | NO. | ADE+ | (%) | NO. | ADE+ | (%) | NO. | ADE+ | (%) |
| 0-4 | 16 | 0 | 0 | 24 | 0 | 0 | 40 | 0 | 0 |
| 5-9 | 14 | 5 | 36 | 27 | 6 | 22 | 41 | 11 | 27 |
| 10-19 | 39 | 9 | 23 | 33 | 8 | 24 | 72 | 17 | 24 |
| 20-29 | 29 | 11 | 38 | 33 | 18 | 55 | 62 | 29 | 47 |
| 30-39 | 19 | 5 | 26 | 25 | 13 | 52 | 47 | 18 | 38 |
| 40-49 | 12 | 3 | 25 | 14 | 6 | 43 | 26 | 9 | 35 |
| 50-59 | 10 | 3 | 30 | 12 | 5 | 42 | 22 | 8 | 36 |
| >60 | 3 | 1 | 33 | 5 | 1 | 20 | 8 | 2 | 25 |
| | 142 | 37 | 26 | 173 | 56 | 32 | 315 | 93 | 30 |

ADE = adenolymphangitis, acute filariasis; 4 persons with elephantiasis, 1 female, 3 males

## TABLE 9

## CLINICAL PREVALENCE RATE IN WALATANA, CENTRAL SULAWESI, INDONESIA

| | FEMALES | | | MALES | | | TOTAL | | |
|---|---|---|---|---|---|---|---|---|---|
| AGE | NO. | ADE+ | (%) | NO. | ADE+ | (%) | NO. | ADE+ | (%) |
| 0-4 | 13 | 0 | 0 | 11 | 1 | 9 | 24 | 1 | 4 |
| 5-9 | 31 | 11 | 35 | 27 | 9 | 33 | 58 | 20 | 34 |
| 10-19 | 57 | 19 | 33 | 45 | 26 | 58 | 102 | 45 | 44 |
| 20-29 | 30 | 3 | 10 | 28 | 19 | 68 | 58 | 22 | 38 |
| 30-39 | 33 | 9 | 27 | 27 | 11 | 41 | 60 | 20 | 33 |
| 40-49 | 15 | 2 | 13 | 26 | 17 | 65 | 41 | 19 | 46 |
| 50-59 | 5 | 3 | 60 | 8 | 3 | 38 | 13 | 6 | 46 |
| >60 | 2 | 0 | 0 | 4 | 2 | 50 | 6 | 2 | 33 |
| | 186 | 47 | 25 | 176 | 88 | 50 | 362 | 135 | 37 |

ADE = adenolymphangitis, acute filariasis; 2 persons with elephantiasis, 0 female, 2 males

## TABLE 10

## ADVERSE REACTIONS TO LOW DOSE DEC MASS TREATMENT IN WALATANA

| Adverse Reaction Wk: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Fever | 34 | 24 | 13 | 12 | 7 | 3 | 0 | 1 | 0 | 0 |
| Headache | 15 | 11 | 5 | 2 | 6 | 3 | 0 | 1 | 0 | 0 |
| Nausea | 23 | 7 | 1 | 2 | 3 | 2 | 1 | 0 | 0 | 0 |
| Vomiting | 22 | 7 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymphangitis | 12 | 2 | 8 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Lymphadenitis | 4 | 1 | 0 | 5 | 9 | 8 | 3 | 2 | 0 | 0 |
| Abscess | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymphedema | 4 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

The drug compliance rate was excellent, yet side effects to DEC treatment very mild.

### III) TREATMENT WITH DEC.

Low dose DEC treatment was implemented in Walatana. This village was chosen by

the flip of a coin. Mantikole became the control village. The DEC dose was 100 mg DEC per week, given to all villagers 10 years of age or over, and half this dose to children less than 10 years of age. Mantikole villagers received a placebo treatment. The treatment was recorded in the treatment cards, and side affects were monitored and symptomatically treated.

a) 25 villagers in Walatana were trained to distribute DEC and record the adverse reactions of the drug.

b) Each drug administrator distributed DEC to 11 families.

c) The population of Walatana was 947 persons. A total of 830 persons received DEC weekly, 607 persons received 1 tablet of 100 mg and 223 half a tablet.

d) Excluded from treatment were 117 persons. They were 13 pregnant and 51 nursing women, as well as 53 babies below 2 years of age.

e) The drug compliance rate for the first 10 weeks of treatment was 98%.

f) Side reactions were mild and of short duration.

g) Major general reactions were fever, headache, nausea and vomiting. Local reactions were lymphangitis, lymphadenitis, lymphedema and abscess (Table 10).

**Accomplishment of objective (3)**

**Longitudinal determination of *B. malayi* ATP over a two year period after DEC treatment.**

To determine the ATP, i.e. the average number of *B. malayi* infective larvae received by inhabitants of each village over a one year period, landing collections of mosquitoes, selection and counting of the vector, *Anopheles barbirostris*, and counting of vectors with, and worms of, each larval stage were performed in each of the villages. The results are presented in Table 11 (village of Mantikole) and Table 12 (Walatana). The relative densities of *An. barbirostris* in Walatana were higher than those of Mantikole throughout the three year period.

## TABLE 11

### LANDING COLLECTIONS OF MOSQUITO VECTORS AND THEIR INFECTION RATES IN MANTIKOLE FROM OCTOBER 1993 TO JUNE 1994

| | OCT | NOV | DEC | JAN | FEB | MAR | APR | MAY | JUN | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| Mosq- Collected | 5712 | 6549 | 8997 | 6850 | 4244 | 3698 | 3278 | 5877 | 4310 | 49515 |
| *A. barbirostris* | 198 | 240 | 360 | 677 | 486 | 495 | 1131 | 871 | 653 | 5111 |
| Infect. *A. barbirostris* | 15 | 12 | 21 | 11 | 6 | 10 | 25 | 19 | 15 | 134 |
| Mosq with L1/No L1 | 7/17 | 5/11 | 8/17 | 2/9 | 1/3 | 4/26 | 14/29 | 7/17 | 3/7 | 51/136 |
| Mosq with L2/No L2 | 4/8 | 3/7 | 6/9 | 4/8 | 1/2 | 2/7 | 3/3 | 6/9 | 5/12 | 34/65 |
| Mosq with L3/No L3 | 4/14 | 4/9 | 7/15 | 5/25 | 4/16 | 4/10 | 8/27 | 6/13 | 7/17 | 49/146 |

## TABLE 12

### LANDING COLLECTIONS OF MOSQUITO VECTORS AND THEIR INFECTION RATES IN WALATANA FROM OCTOBER 1993 TO JUNE 1994

| | OCT | NOV | DEC | JAN | FEB | MAR | APR | MAY | JUN | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| Mosq- Collected | 2661 | 3885 | 4861 | 3677 | 2383 | 2403 | 2481 | 3351 | 3731 | 29439 |
| *A. barbirostris* | 218 | 494 | 1170 | 672 | 380 | 379 | 985 | 616 | 789 | 5003 |
| Infect. *A. barbirostris* | 2 | 3 | 10 | 3 | 4 | 6 | 5 | 3 | 6 | 42 |
| Mosq with L1/No L1 | 1/1 | 3/8 | 6/10 | 3/3 | 1/4 | 2/2 | 2/9 | 0 | 1/1 | 19/38 |
| Mosq with L2/No L2 | 0 | 0 | 2/2 | 0 | 1/3 | 0 | 1/3 | 1/1 | 4/8 | 9/17 |
| Mosq with L3/No L3 | 1/2 | 0 | 2/2 | 0 | 2/4 | 4/8 | 2/2 | 2/4 | 1/2 | 14/24 |

The monthly infection and infective rates are depicted in Table 13, and a summary of entomological data over the 3-year period is presented in Table 14. The infection rates in Walatana decreased significantly from 3.5% to 0.8% while those in Mantikole remained more or less the same. The infective rates in Walatana decreased from 1.7% to 0.3% while those in Mantikole remained stable. The worm burden per infective mosquito decreased from 5.0 to 1.7 in Walatana while those in Mantikole decreased just slightly.

The annual number of infective bites in Walatana decreased from 652 to 56 while those in Mantikole decreased slightly. The number of L3S a person received per year (ATP)decreased from 3280 in 1991-92 to 96 in 1993-94, a decrease of 97%. In the village of Mantikole (inhabitants not treated), the ATP also decreased, form 1602 in 1991-92, to 584 in 1993-94, a decrease of 67%.

The infective larvae were tested against the stage and species specific monoclonal antibodies and they were found to be *B. malayi* (Table 15).

**TABLE 13**

**MONTHLY INFECTION AND INFECTIVE RATES OF MOSQUITO VECTORS IN MANTIKOLE AND WALATANA FROM OCTOBER 1993 TO JUNE 1994**

| MANTIKOLE | OCT | NOV | DEC | JAN | FEB | MAR | APR | MAY | JUN | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Infection Rates(%) | 7.6 | 5.0 | 5.8 | 1.6 | 1.2 | 2.0 | 2.2 | 2.2 | 2.3 | 2.6 |
| Infective Rates(%) | 2.0 | 1.7 | 1.9 | 0.7 | 0.8 | 0.8 | 0.7 | 0.7 | 1.1 | l.0 |

| WALATANA | OCT | NOV | DEC | JAN | FEB | MAR | APR | MAY | JUN | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Infection Rates(%) | 0.9 | 0.6 | 0.9 | 0.5 | 1.0 | 1.6 | 0.5 | 0.5 | 0.8 | 0.8 |
| Infective Rates(%) | 0.5 | 0 | 0.2 | 0 | 0.5 | 1.0 | 0.2 | 0.3 | 0.1 | 0.3 |

**TABLE 14**

**COMPARISON OF *ANOPHELES BARBIROSTRIS* INFECTION AND INFECTIVE RATES IN MANTIKOLE AND WALATANA BETWEEN OCTOBER 1993 AND JUNE 1994**

| | MANTIKOLE | | | WALATANA | | |
|---|---|---|---|---|---|---|
| | 91-92 | 92-93 | 93-94 | 91-92 | 92-93 | 93-94 |
| Relative density of *A. barb.* | 5.6% | 12% | 10% | 18.6% | 29% | 17% |
| Infection rates | 2.5% | 3.2% | 2.6% | 3.5% | 1.4% | 0.8% |
| Infective rates | 1.3% | 1.2% | 1.0% | 1.7% | 0.5% | 0.3% |
| Nº L3/ infected mosq. | 4.4 | 3.2 | 3.0 | 5.0 | 1.7 | 1.7 |
| Infective bites/year | 364 | 400 | 196 | 652 | 205 | 56 |
| L3 a person received per year | 1602 | 1260 | 584 | 3280 | 356 | 96 |

**TABLE 15**

**PERCENTAGE OF STAINED LARVAE IN ELISA USING $D_1E_5$ MONOCLONAL ANTIBODY AGAINST L3 RECOVERED FROM INFECTED *A. BARBIROSTRIS***

| MANTIKOLE | | | WALATANA | | |
|---|---|---|---|---|---|
| 91-92 | 92-93 | 93-94 | 91-92 | 92-93 | 93-94 |
| 88 | 90 | 91 | 89 | 87 | 92 |

**50% of the recovered L3 were tested.**

Accomplishment of objective (4)

**Reassessment of microfilaria and adenolymphangitis rates of the villagers at the end of the study period. DEC treatment of inhabitants of the village given placebo.**

Microfilaria and adenolymphangitis rate determinations in the two villages have now been completed. The data are summarized in Table 16. The microfilaria rate in Walatana decreased from 21% initially to 1% at the end of the third year. The microfilaria rate in Mantikole remained more or less the same. The adenolymphangitis rate in Walatana decreased from 37% to 7%. The adenolymphangitis rate decreased in Mantikole just slightly. The side effects were minimal and of short duration. At the end of the third year the villagers in Mantikole were treated with DEC. Although the microfilaria rate decreased very significantly in the village of Walatana, it is noteworthy that it was not reduced to zero. This may contribute to explain why the ATP was also not reduced to zero in the village.

**TABLE 16**

**MICROFILARIA AND ADENOLYMPHANGITIS RATES IN MANTIKOLE AND WALATANA BEFORE AND AFTER DEC TREATMENTS**

| | MANTIKOLE | | WALATANA | |
|---|---|---|---|---|
| | Before | After Placebo | Before | After DEC |
| Mf rates | 16% | 13% | 21% | 1.0% |
| Adenolymph. rates | 30% | 21% | 37% | 7% |

## IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The location of the center of gravity of this collaborative project was the University of Indonesia and its field outposts in Central Sulawesi. Dr. Partono achieved an extraordinary collaborative and organizational feat with his field entomologists and a large group of mosquito collectors and local DEC administrators. It is through his scientific and organizational talent that this project was finished successfully. We are very pleased with the results. Although we were not able to interrupt transmission of *B. malayi* in Walatana, we were able to diminish transmission very significantly. Hence, we hope that our approach will be seriously considered as an inexpensive alternative for the control of transmission of Malayan filariasis.

The University of Indonesia is one of the oldest in the country. It has been

actively involved in field and laboratory studies of parasitic diseases, especially those of medical and public health importance, since the early nineteen forties. Most of the staff are government employees. It is the aim of the Department of Parasitology that as many of its scientists as possible be trained in modern biotechnology. Examples of this effort are collaborations with the Department of Pure and Applied Biology of Imperial College, London, U.K., the Molecular Parasitology Group of New England Biolabs, Beverly, Massachusetts, the Department of Biology of Smith College, Northampton, Massachusetts, and the Department of Parasitology of the Tulane University Medical Center, Covington, Louisiana. Young members of the Department have spent training periods in these institutions, during which they studied monoclonal antibody technology and molecular biology techniques as applied to diagnostic purposes.

This endeavor is being greatly strengthened by showing, as we have with this project, that one of the products of modern biotechnology research, i.e. a monoclonal antibody-based diagnostic ELISA, can make a direct and unique contribution to answering an epidemiological question of relevance to Indonesia and other developing countries.

## PROJECT ACTIVITIES/OUTPUTS

A publication summarizing the data in this report is in preparation.

## PROJECT PRODUCTIVITY

The project accomplished all of the proposed objectives. The working hypothesis, that transmission of filariasis due to *B. malayi* can be interrupted by chemotherapy alone, was proven in part, in that the Annual Transmission potential of *B. malayi* was significantly diminished.

## FUTURE WORK

No future work is planned at this point.