ISSN 1411 – 3481

CONTROLLING *Aedes aegypti* POPULATION AS DHF VECTOR WITH RADIATION BASED-STERILE INSECT TECHNIQUE IN BANJARNEGARA REGENCY, CENTRAL JAVA

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Diterima: 19-09-2012
Diterima dalam bentuk revisi: 25-10-2012
Disetujui: 28-01-2013

ABSTRACT

CONTROLLING *Aedes aegypti* POPULATION AS DHF VECTOR WITH RADIATION BASED-STERILE INSECT TECHNIQUE IN BANJARNEGARA REGENCY, CENTRAL JAVA. The control program of dengue hemorrhagic fever (DHF) in Indonesia is still a problem due to the incomplete integrated handling. Sterile insect technique (SIT) for *Aedes aegypti* as DHF vector was considered as a potential strategy for controling the DHF. A preliminary survey was carried out to to determine the characteristic of *A. aegypti* population in the study site before the implementation of SIT. The implementation of radiation based-SIT was carried out in Krandegan and Kutabanjar Villages of Banjarnegara Regency, Central Java which involved 99 houses. One hundred gamma rays irradiated male mosquitoes were released to each house up to five times. The eggs, larvae and adult mosquitoes were collected using ovitrap and weekly observed. The initial population density of *A. aegipty* in the studied area was obtained to be 6 mosquitoes per house with the mean index of house was 15.86 % and the mean sterility of sterilized mosquitoes was 79.16 %. The SIT effectively reduced *A. aegipty* population after the fifth release of irradiated mosquitoes into the houses. It can be assumed that the SIT was effective in controlling DHF vector in the studied area, nevertheles, it will be more effective if it is combined with other handling techniques.

Keywords: DHF, *Aedes aegypti*, SIT, radiation

ABSTRAK

PENGENDALIAN POPULASI *Aedes aegypti* SEBAGAI VEKTOR DBD DENGAN TEKNIK SERANGGA MANDUL BERBASIS-RADIASI DI KABUPATEN BANJARNEGARA, JAWA TENGAH. Program pengendalian demam berdarah dengue (DBD) di Indonesia masih terkendala karena tidak lengkapnya penanganan yang terpadu. Teknik serangga mandul (TSM) untuk *Aedes aegypti* sebagai vektor DBD dianggap sebagai strategi yang potensial dalam mengendalikan DBD. Sebelum implementasi TSM, sebuah survey pendahuluan dilakukan untuk menentukan karakteristik populasi *A. aegypty* di lokasi yang akan dikaji. Penerapan TSM berbasis radiasi dilakukan di Desa Krandegan dan Kutabanjar, Kabupaten Banjarnegara, Jawa Tengah dengan melibatkan 99 rumah. Seratus nyamuk jantan mandul hasil iradiasi gamma dilepaskan hingga 5 kali pada setiap rumah di area penelitian. Telur, larva dan nyamuk dewasa dikumpulkan menggunakan ovitrap dan jumlahnya diobservasi setiap minggu. Kepadatan populasi awal nyamuk *A.aegipty* di area studi diperoleh sebanyak 6 nyamuk per rumah dengan indeks rata-rata rumah adalah 15,86 % dan sterilitas nyamuk adalah 79,16 %. Hasil penelitian menunjukkan bahwa setelah pelepasan ke lima, TSM secara efektif dapat menurunkan populasi *A. aegipty* dan diasumsikan bahwa TSM efektif mengendalikan vector DBD di area studi, namun akan lebih efektif jika dikombinasikan dengan upaya penanganan lainnya.

Kata kunci: DBD, *Aedes aegypti*, TSM, radiasi

1. INTRODUCTION

Dengue hemorrhagic fever (DHF) is generally found in tropical and sub tropical areas. World Health Organization (WHO) estimates that nearly 50 million of dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries including South East Asia Region (1,2). In Indonesia, the number of reported DHF cases was increased in 2004 and reached a plateau between 2007 and 2009. In 2004 there were 30 provinces affected by the disease with totally 78,690 cases and 954 deaths. It was increased in 2010 with 150,000 cases, 1317 deaths, and the case-fatality rate was approximately 1%. Almost 60% of Indonesian people are living in Java, which is most severely afflicted by periodic outbreaks of dengue disease (3,4). However, the disease is also endemic in many large cities and small towns throughout the country and has also spread to certain smaller villages, where population movement and density are high.

The DHF vector borne disease can still not completely be handled, even in some areas with the outbreaks (5). Dengue fever is a relatively common problem in Indonesia and periodically reaches epidemic proportions in all parts of the country, usually every 4-5 years. The outbreak of dengue fever is most common during the rainy season (November – May) as the mosquito requires clean standing water to reproduce. Dengue fever is an endemic disease both in Java and outside Java which is transmitted from sick to healthy people through the bite of a *flavivirus* infected *Aedes aegypti* mosquito. Although the eradication of *A. aegypti* population is often conducted, the significant results have not yet achieved. Many factors are thought to be responsible for the re-emergence of DHF, including major global demographic changes, also the worsening of health care systems and mosquito control programmes (6). Even after more than 60 years of research, a licensed vaccine against the virus is still elusive, and community-based vector control programmes have not been very successful (7).

Sterile insect technique (SIT) is a relatively new and reported to be a potential vector control measures, effective, species-specific and compatible with other control measures. The basic principle of SIT is very simple, i.e. kills insect by the insect itself (autocidal technique). The SIT includes a sequence of activities that are interlinked each other, covering from maintenance of insects in the laboratory, irradiation for insect sterilization, population dynamics and release into the field (6,8). The released sterile male insects will compete with normal males in copulated with female insects. If the release of sterile male insects is performed continuously, then the insect populations at the release site will be low (9,10). The SIT implementation will be better if it is combined with other integrated vector controls such as insecticides utilization, good environmental sanitation, good water management, also the use of predator nets and netting installation in the house.

There are three types of *Aedes* mosquitoes that known as transmitter of dengue disease in Indonesia, i.e. *A. aegypti, A. albopictus* and *A. scutelaris*. Of these, *A.*

aegypti has the greatest role in transmission of the disease (8). The conventional vector control is still less successful, therefore SIT is considered to be an alternative to control the vector since the SIT is a specific biological vector control technique and affects only the target species. In the SIT, the sterile insects are gradually and continuously released to the field that the *A. agepty* will be eradicated, and it is expected that at the 5-th generation the mosquito populations will be depleted (9,11).

The first study of SIT in restricted area was conducted in 2010 by releasing the sterilized male of *A. aegypti* in Pasar Jum'at Nuclear Research Center, South Jakarta. The study result showed that the population of dengue was reduced after several times release of the sterilized male *A. aegipty* (12,13). In the present study, irradiation-based SIT was applied to evaluate the effectiveness of the technique in controlling the population of *A. aegypti* as dengue vector in Krandegan and Kutabanjar villages of Banjarnegara Regency, Central Java.

2. MATERIAL AND METHODS

2.1. Study Area

Krandegan and Kutabanjar villages are located in Banjarnegara Regency, Central Java Province of Indonesia, at 7° 12' - 7° 31' South Latitude and 109° 29' - 109° 45'50" East Longitude (Fig. 1). The Banjarnegara regency occupies an area of 106,970.997 hectares or 3.10% of the Central Java Province. In 2012, the total population of Banjarnegara Regency was 823,110 people (14).

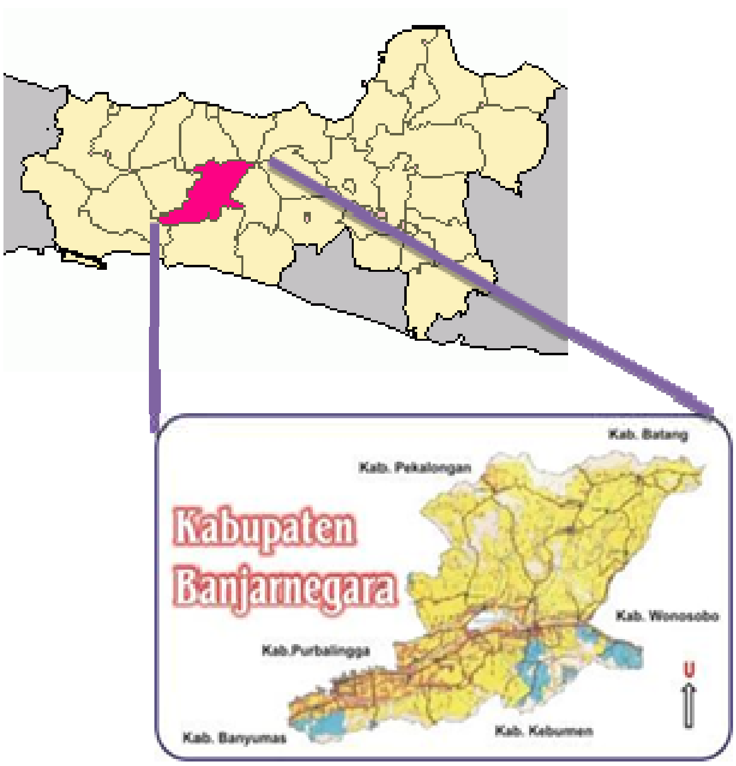

Figure 1. The study area of DHF vectors control (15).

2.2. Laboratory Mass Rearing of Mosquito

Aedes aegypti was reared in the insectariant of the Center for Application of Isotope and Radiation Technology, BATAN at temperature of 22 – 25 °C and relative humidity of 70%. The eggs colony of *A. aegypti* that stuck to the filter paper was soaked into the water in a plastic trays of 25 × 30 × 5 cm^3 of size. The hatched larvae were then fed with pellet that made of dog or cat diet before they become pupae.

The adult mosquitoes emerged from pupae were separated between male and female mosquitoes using a glass aspirator (vacuum). These adults mosquitoes were maintained on distilled water for 12–15 hours prior to blood feeding on a restrained guinea pig for up to 45 minutes depending on feeding rate (90% of females typically feed within 20 minutes). A few hours post-feeding, unfed females were removed. Egg bank must be conducted continuously to get a sufficient stock of mosquitoes during the study. For continuous stock, *A. aegypti* eggs stick to the filter paper was stored in dry condition in desiccators.

2.3. Production of Sterile Male Mosquitoes

Hundred male mosquitoes contained in a 100 cc size plastic vial were irradiated using Gamma Cell 220 machine in IRPASENA Irradiator of Center for Application of Isotope and Radiation Technology, BATAN (Fig. 2) with infertility dose of 70 Gy at 380 Gy/hour of dose rate. According to the previous study, the irradiation dose of 70 Gy resulted the sterility percentage of 100% and mating competitiveness of 0.31 (13).

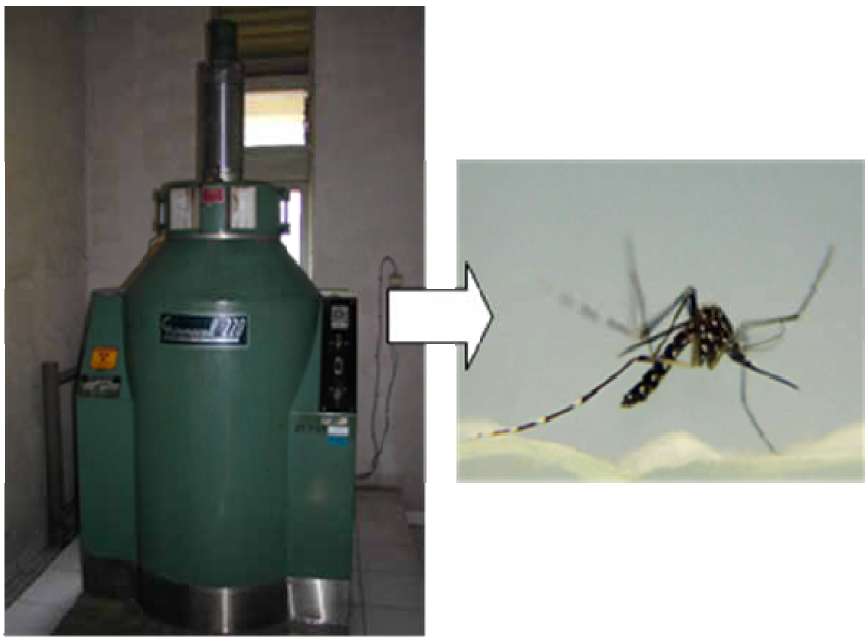

Figure 2. The adult mosquitoes irradiation using Gamma Cell 220 machine in IRPASENA Irradiator.

The mating competitiveness was calculated using equation [1] for following mating combinations:

1) ♂N : ♀ N = 1 : 1
2) ♂R : ♀ N = 1 : 1
3) ♂ R: ♂ N : ♀ N = 1 : 1 : 1
4) 3 ♂ R : ♂ N : ♀ N = 3 : 1 : 1

Irradiated male was coded as R and non irradiated male and female was coded as N.

$$\text{Mating Competitiveness} = \frac{(Ha\text{-}E) / (E\text{-}Hs)}{\text{S/N Actual (control)}} \quad [1]$$

Where, Ha = percentage of the hatched eggs for control (1♂N : 1 ♀N); Us = percentage of the hatched eggs from (l ♂R : 1 ♀N); N = number of normal males (treated); S = number of irradiated males; and E = percentage of the hatched eggs from (3 ♂R : 1 ♂N : 1 ♀N).

The mosquitoes were fed with 10% honey/sugar solution after irradiation and followed by sterile male mosquitoes release to the location.

2.4. Natural Vector Population Dynamics Studies on The Site

A preliminary survey was carried out to estimate the points where the *A. aegypti* breeding was endophilic. The survey to the initial population was conducted every Wednesday weekly for five weeks, started in March 4, 2011, to determine the characteristic of *A.aegypti* population in the site before the implementation of SIT. Ovitrap devices were placed in the living room of the houses invlolved in this study (Fig 3A). An ovitrap consists of a black polyethylene cup of 473 ml capacity with 11 cm high, 6.5 cm and 9 cm in the diameter of the bottom and the upper cup, respectively. A strip of red colour, heavy-weight paper was attached to the inner side of the cup with a paper clip. The ovitrap was filled with water until reach 2.5 cm from the top. The black ovitrap attracts female mosquitoes to lay their eggs. The mosquito larvae emerged from the eggs were maintained and observed every week during the study until they become adult. The observations were made on the number of adult mosquitoes captured in each ovitrap. The ovitrap breeding data collected every week was then analysed to estimate the initial population of *A. Aegypti,* the number of sterilized mosquitoes that will be released, and the level of SIT success at the end of the program. The mosquitoes population dynamic data was also used as control data.

2.5. The Release of Sterile Male Mosquitoes

Sterile male mosquitoes were released at the study location every week near the water collection place in the house (Fig. 3a, 3b, 3c). The location of the house does not need to be isolated since the mosquitoes fly in limited distance of an area. The number of sterile male released on the location to be controlled was at least nine times of the amount of natural populations based on the results of the survey on natural population dynamics. One hundred sterile mosquitoes was fixed to be released.

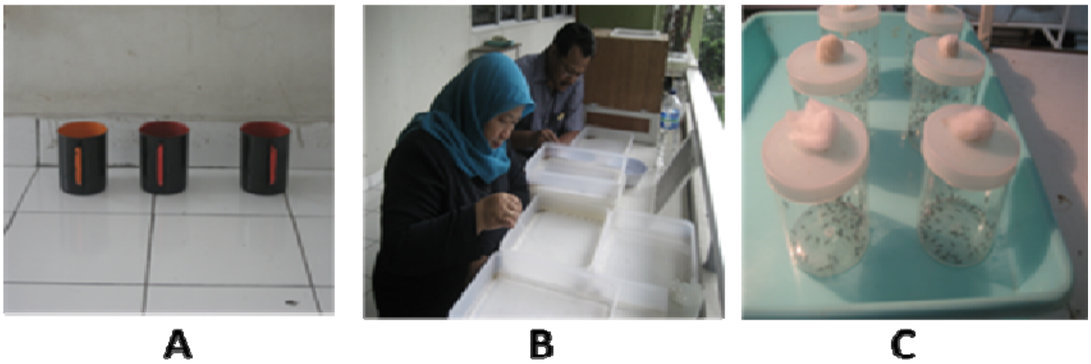

Figure 3. A. Ovitrap installation in areas that will be controlled; B. Manual selection for irradiated male pupae, C. Sterilized male mosquitoes in special cups to be deployed to the location of study.

2.6. The SIT Analysis

Ovitraps were placed at the release site in the living room of the houses (Fig 3a). The adult mosquitoes trapped in the ovitrap were observed and analyzed every week by counting the larvae and eggs found in the ovitrap. The success or effectiveness of SIT was characterized by the declining number of *A. aegypti* populations caught in the ovitrap. The effectiveness of SIT was calculated as a percentage of the decrease of mosquitoes population after release compared to initial population.

3. RESULTS AND DISCUSSION

Based on the Ministry of Public Health, there was an endemic of DHF in Krandegan and Kutabanjar villages in the last three years from 2008 to 2010 (15). Therefore, 99 houses in those both villages were enrolled in this study based on the agreement between the house hold and the team of study. The result of survey on natural vector population dynamics at the site showed that the density of *A. aegipty* was 6 mosquitoes per house based on the ovitrap observation located in 99 houses at eight locations (Table 1).

Table 1. Initial population of mosquitoes before implementation of SIT in Banjarnegara District, Central Java

Location	Number of house	Number of larvae	Mean of larvae/house
I	11	18	18/11 = 1.64
II	8	8	8/8 = 1.00
III	12	9	9/12 = 0.75
IV	10	146	146/10 = 14.60
V	11	100	100/11 = 9.09
VI	15	266	266/15 = 17.73
VII	21	35	35/20 = 1.67
VIII	11	14	14/11 = 1.27
Total	**99**	**596**	**596/99 = 6.02**

Using the combination of irradiated male (R) : non irradiated male (N) : non irradiated male (N) as 3:1:1, the release of sterile male mosquitoes of *A. aegypti* in Krandegan and Kutabanjar villages, Banjarnegara Regency of Central Java showed that the mean index of house (percentage of house with mosquitoes finding compared to all houses) was 15.86%. The percentage of fertility that determined by counting the larvae emerged from eggs was 20.84%, so that the effectiveness of sterilized mosquitoes was 79,16% (Table 2).

Table 2. Ratio of larvae to eggs obtained from 12-22 positive ovitraps of 99 houses under study, percentage of fertility and house index

House number	Times of mosquito releases into the house				
	1-st	**2-nd**	**3-rd**	**4-th**	**5-th**
1	8/80	0/5	3/11	20/59	14/48
2	2/3	4/64	6/6	9/99	26/47
3	1/17	2/18	0/9	3/40	0/101
4	2/9	19/37	7/258	4/9	24/28
5	0/6	11/32	4/157	4/25	0/41
6	8/44	0/70	0/3	0/4	5/24
7	6/22	0/66	2/11	0/16	32/47
8	0/6	0/22	52/216	16/66	24/34
9	13/34	4/74	7/30	1/34	11/15
10	11/16	1/44	55/85	3/10	0/17
11	4/24	2/87	0/6	51/67	5/35
12	5/30	3/39	7/44	1/28	17/51
13	1/21	1/25	3/7	-	4/22
14	0/57	0/37	2/8	-	4/28
15	-	0/45	54/114	-	-
16	-	0/37	15/20	-	-
17	-	-	4/8	-	-
18	-	-	16/76	-	-
19	-	-	0/4	-	-
20	-	-	3/14	-	-
21	-	-	7/21	-	-
22	-	-	38/45	-	-
House with positive ovitrap	**14**	**16**	**22**	**12**	**14**
House index	**14/99 (14.1%)**	**16/99 (16.2%)**	**22/99 (22.2%)**	**12/99 (12.1%)**	**14/99 (14.1%)**
	Mean house index = 15.86 %				
Total larvae/egg (% fertility)	61/369 (16.53)	47/702 (6.70)	285/1,153 (24.72)	112/457 (24.51)	166/538 (30.86)
	Mean = 671/3,210 = 20.84 %				

During the study the sterility of the mosquitoes in the area after SIT application was evaluated by determining the number of larvae emerged from the eggs. The ratio of the amount of larvae compared to the eggs was varied, ranging between 0 larvae per 101 eggs (0%) at the fifth release as the highest ovitrap index and 6 larvae per 6 eggs (100%) at the third release as the lowest ovitrap index (Table 2).

The study on radiation based-SIT implementation to control *A. aegepty* is the first in a community in Indonesia and still faced some obstacles that should be solved together. The SIT was first used successfully in 1958 in Florida to control screwworm fly (*Cochliomya omnivorax*) (16). About 50 million flies were released per week over an 18 months period, in a total of 2 billion flies over 85,000 square mile area. The pest was eradicated after this period of application, in which 40 tons of ground meat and 20 aircrafts were required each week to release the sterile flies. The total cost was about US$ 10,000,000.00. Much researches on the application of SIT to mosquitoes were carried out about 30 years ago, especially in India and El Salvador. Unfortunately this technique virtually ends in the mid-1970s, not because of the method was technically failure, but because of political problems in India and intensifying civil wars in Central America (16). There is now a revival of interest especially in the use of transgenesis to improve sex separation, so that only non biting males are released but to ensure that their female progeny die without the need for radiation or chemosterilization (17). Therefore, with some improvements through the use of currently available transgenic technologies, SIT could become a mainstay for public health control of specific vector-borne diseases. A number of mathematical models have been done to assist the effectiveness of the SIT (18).

Controlling the mosquito as DHF vector can be done by several methods. A study in Australia found that injection of harmless bacterium called *Wolbachia* into mosquitos in affected region could prevent the mosquitoes from passing the dengue fever virus along to their own offspring (19). Other study used mosquitoes that are genetically engineered kill their own children (20). Goddard J. (21) stated that the best mosquito control program including *A. aegypti* is an integrated program that includes point source reduction of breeding areas, routine larviciding in those breeding areas that can not be eliminated, and adulticiding only when necessary. In this present day of environmental consciousness, municipal leaders must try to use integrated methods of mosquito control and not just routine spraying with a fogging truck.

4. CONCLUSION

The implementation of radiation based-SIT in the area with *A. aegypti* population density of 6 mosquitoes / house showed that the radiation based-SIT can effectively reduce 79.16% population of mosquito with the mean index of house was 15.86% after the fifth release of sterile male mosquitoes. Therefore, it is hoped that this technique was also effective in controlling DHF vector in the villages. It will be more effective if this SIT is combined with other vector handling.

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