CHAPTER I
INTRODUCTION

1. Background and Rationale

The most important vector of dengue virus is the mosquito *Ae. aegypti* which should be the main target of control activities. There is reliable evidence that they represent an epidemiologically significant role in the transmission of dengue infections (1).

1.1 Dengue Virus (DEN)

Dengue is the most important aboviral infection of man (2), approximately 100 million cases annually and 2.5 billion people at risk (3). In Thailand, 46,829 DHF cases have been reported in 2006 and 59 cases were died (4). DEN is a member of the family *Flaviviridae*, genus *Flavivirus*. There are 4 dengue virus serotypes (DEN1-4) (5, 6, 7, 8) contain a positive sense RNA genome (11Kb) that encodes three structural (C-preM-E) and seven nonstructural proteins (NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5) (7). The three structural genes are translated into proteins that form the shell of mature viral particles. Non-structural proteins are assumed to be important during different stages of virus replication and maturation in the host cell. The epidemiology of the 4 dengue virus serotypes is similar all have worldwide distribution in the tropics and are maintained in most tropical urban centres in a mosquito-human-mosquito cycle (3, 5, 7). The principal mosquito vector is *Ae. aegypti* which was originally indigeous to North Africa, but has spread throughput other parts of the tropical world via ships or other commercial vessels. This species become highly adapted to living in intimate association with humans and is a highly efficient epidemic vector in urban setting. DNV is transmitted from human to human by the bites of female mosquito (3), *Ae. aegypti* can transmit dengue either immediately by a change of host or after an incubation period of 8-10 days during which time the virus multiplies in the salivary gland of the mosquito. DNV infection causes a spectrum of clinical illness, ranging from inapparent infection to mild non-specific viral syndrome to classical dengue fever to severe and fatal haemorrhagic disease (dengue haemorrhagic fever, DHF).

The lack of specific treatment for dengue virus and unavailability of effective vaccine against the virus (8), the interruption of pathogen transmission by mosquito control provides the only effective approach to the control of dengue infection. Chemical control used to be the primary strategy for controlling mosquito borne diseases, but concerns about the effect on the environment and human health together with the presence of insecticide in mosquitoes have limited the usefulness of this approach. A possible alternative approach to control dengue is to create mosquito strains that are unable to transmit the virus.

A recombinant double stranded Sinbis (dsSIN) virus containing 567bp antisense RNA fragments directed to the premembrane (prM) coding region of the Dengue type 2 virus (DEN-2 prM RNA) when introduced into mosquitoes could inhibit the replication of dengue type 2 virus within *Ae. aegypti* mosquitoes and thus prevent its transmission (9). The C6/36 (*Ae. albopictus*) cells transfected with dsSIN virus containing antisense DEN-2 prM RNA were completely resistant to DEN-2 challenge (10). Recently, expression of RNA interference against dengue virus type 2 in the midgut of genetically modified *Ae. aegypti* mosquitoes was interrupt the dengue type 2 replication in the transformed mosquitoes (11). Expression of similar molecules in genetically transformed mosquitoes could lead to the production of strains that can be used in the control of transmission of pathogens (12, 13).

Although dengue-resistance mosquito strains have been successfully developed, releasing of the transgenic mosquitoes also faces a number of problems including the fitness cost of these transgenic mosquitoes. *Wolbachia* has seem to be a potential gene driving system genetically manipulated mosquitoes by suppression or replacement of vector populations the population replacement. In this study we would like to determine whether bed bug *Wolbachia* could induce cytoplasmic incompatibility in *Ae. aegypti* mosquitoes, therefore it could be used for population replacement in transgenic *Ae. aegypti* mosquitoes.

1.2 *Aedes aegypti* mosquitoes

Ae. aegypti mosquitoes belong to the family Culicidae and are small fragile insects. They are living in the tropical and sub-tropical regions of the world (14). *Ae. aegypti* is one of the most important mosquito vectors and is commonly recognized to cause dengue and yellow fever epidemics (15).

1.3 Vector control

The most effective means of vector control is environment management, which includes planning, organization, carrying out and monitoring activities for the modification or manipulation of environmental factors with view to preventing or reducing vector propagation and human-vector-pathogen contact (1). The approaches used in vector control can be classified into four main groups, environmental, chemical, biological and genetic control. For the successful of mosquito control strategies, a number of factors must be considered including the economic and political situation of each country and along with the ecology of different mosquito species (16).

1.3.1 Environmental Control

Environment management methods to control *Ae. aegypti* and *Ae. albopictus* and reduce human-vector contact include the improvement of water supply and storage, solid waste management and the modification of man-made larval habitats (1). This method is particularly applicable to the urban dwelling *Ae. aegypti* mosquitoes where control has been achieved through education of the local populations in basic domestic hygiene. Environment management should focus on the destruction, alteration, disposal or recycling of containers and natural larval habitats that that produce the greatest number of adult *Ae. aegypti* mosquitoes in each community (1). For example, collection and disposal the water impoundment, good water fluctuation and marginal drainage.

Environmental control measures have an advantage in that they are effective in the long term and have relatively little adverse environment

impact. However the successful of this method requires the co-operation of the whole community.

. 1.3.2 Chemical Control

Chemical control is especially well suited to short term use during epidemic outbreaks to reduce the effective population size of the vector. However, there are criticizes for environment and social reasons associated with its long term including the hazards to the human population through direct (inhalation or skin contact) and indirect contact (contaminated food). There are not species specific, hence destroy most insects and the development or resistance of insecticide in vectors. Current methods for applying insecticides include larvicide application, perifocal treatment and space spraying.

Larvicidal or "focal" control of *Ae. aegypti* is usually limited to containers maintained for domestic use that cannot be eliminated. Three larvicides can be used to treat containers that hold drinking-water: 1% temephos sand granules, the insect growth regulator methoprene in the form of briquetters, and BTI (*Bacillus thuringiensis* H-14). All these larvicides have extremely low mammalian toxicity, and properly treated drinking-water is safe for human consumption (1).

Perifocal treatment involves the use of hand or power sprayers to apply wettable power or emulsifiable-concentrate formulations of insecticide as a spray to larval habitats and peripheral areas. This will destroy existing and subsequent larval infestations in containers of non-potable water, as well as kill the adult mosquitoes that frequent these sites. This method can be used to treat containers that are preferred by *Ae. aegypti*, whether or not they hold water. The internal and external walls of containers are sprayed until they are covered by a film of insecticide; spraying is also extended to cover any wall within 60 cm of the container. The surface of non-potable water in containers is also treated (1).

Space spraying is the spreading of microscopic droplets of insecticide in the air to kill adult mosquitoes and is used in emergency situations when an outbreak of dengue fever is already I progress (1).

1.3.3 Biological control

Interventions based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce the numbers of *Ae. aegypti* or *Ae. albopictus* remain largely experimental and information on their efficacy is based on the results of small-scale field operations. Larvivorous fish and the biocide *Bacillus thuringiensis* H-14 (BTI) are the two organisms most frequently employed (1).

The advantages of biological control measures include an absence of chemical contamination of the environment and specificity of activity against the target organism and the self-dispersion of some agents into sites that could not be easily treated by other means. The disadvantages of these measures include the expense of rearing the organism, difficulty in their application and rearing and limitation of utility in aquatic sites where temperature, pH and organic pollution meet narrow specifications (16).

1.3.4 Genetic Control

The aim of genetic control is to reduce autocide among the target population. One approach involves the mass release of chemically and radiologically sterilized males. The approach relies on the fact that after release the sterile males will be in vast excess to the indigenous population and will compete for the females. The result would be a massive decrease in the number of offspring produced. But, the sterile males were required as a fully fertile generation could result in the filling of the emptied ecological niche. An alternative concept of genetic control is based on modification of the natural vector to support pathogen development. The transfer of *Wolbachia* infection (transinfection) for applied strategies to affect suppression or replacement is the once strategy to control of vector-borne disease (17).

1.4 Bed bugs

The Cimicidae (bed bug) are obligatory hematophagous ectoparasites of birds, bats and humans (18, 19). Although, the bed bugs are not

medically important or no vector of any pathogens but they can cause distress and infestations to humans (20).

1.5 Bed bugs *Wolbachia*

Wolbachia, rickettsia-like proteobacteria inclusions were observed by microscopy in several cimicid species (18) and modern molecular methods were used to conclusively identify *Wolbachia* symbionts in this species (19). More recently, the molecular evidence from *Wolbachia* specific genes (*ftsZ* and *16S* rDNA) were used to conclusively identify *Wolbachia* symbionts in bed bugs which were infected with *Wolbachia* of the F supergroup. Since, the bed bugs *Wolbachia*-like bacterial inclusions were observed in the gonads, spermalege, gut, Malpighion tubules and hemolymph (19). So, *Wolbachia* from bed bugs may be high density and able to high transmit into offspring.

The successful of genetic modification strategies of vector control to suppression and replacement population are based on intracellular *Wolbachia* bacteria (21). In this study, bed bugs *Wolbachia* were selected for genetic control of *Ae. aegypti* mosquitoes to reduce or block pathogen transmission because these bacteria as an agent that might promote insect speciation and as an applied tool for insect pest and disease control (22).

2. Research questions

2.1 Major research question

Could *Wolbachia* "F-supergroup" be cross transinfection from *C. hemipterus* into *Ae. aegypti* mosquito?

Is the level of *Wolbachia* transinfection to offspring associate with *Wolbachia* density?

2.2 Minor research question

Is the establishment of *Wolbachia* transinfect "F-supergroup" from *C. hemipterus* into *Ae. aegypti* capable to transmit into offspring and nucleotide bases have been change?

3. Objectives

To study effects of *Wolabachia* cross transinfection from bed bug *C. hemipterus* into *Ae. aegypti* by direct microinjection.

To determine whether bed bug (*C. hemipterus*) *Wolabachia* is able to survive in *Ae. aegypti* mosquito.

To determine CI expression of transinfected *Ae. aegypti* mosquito.

To determine whether the *ftsZ* and *16S* rDNA sequences are changed when *Wolbachia* are transfer into the new host.

4. Hypothesis

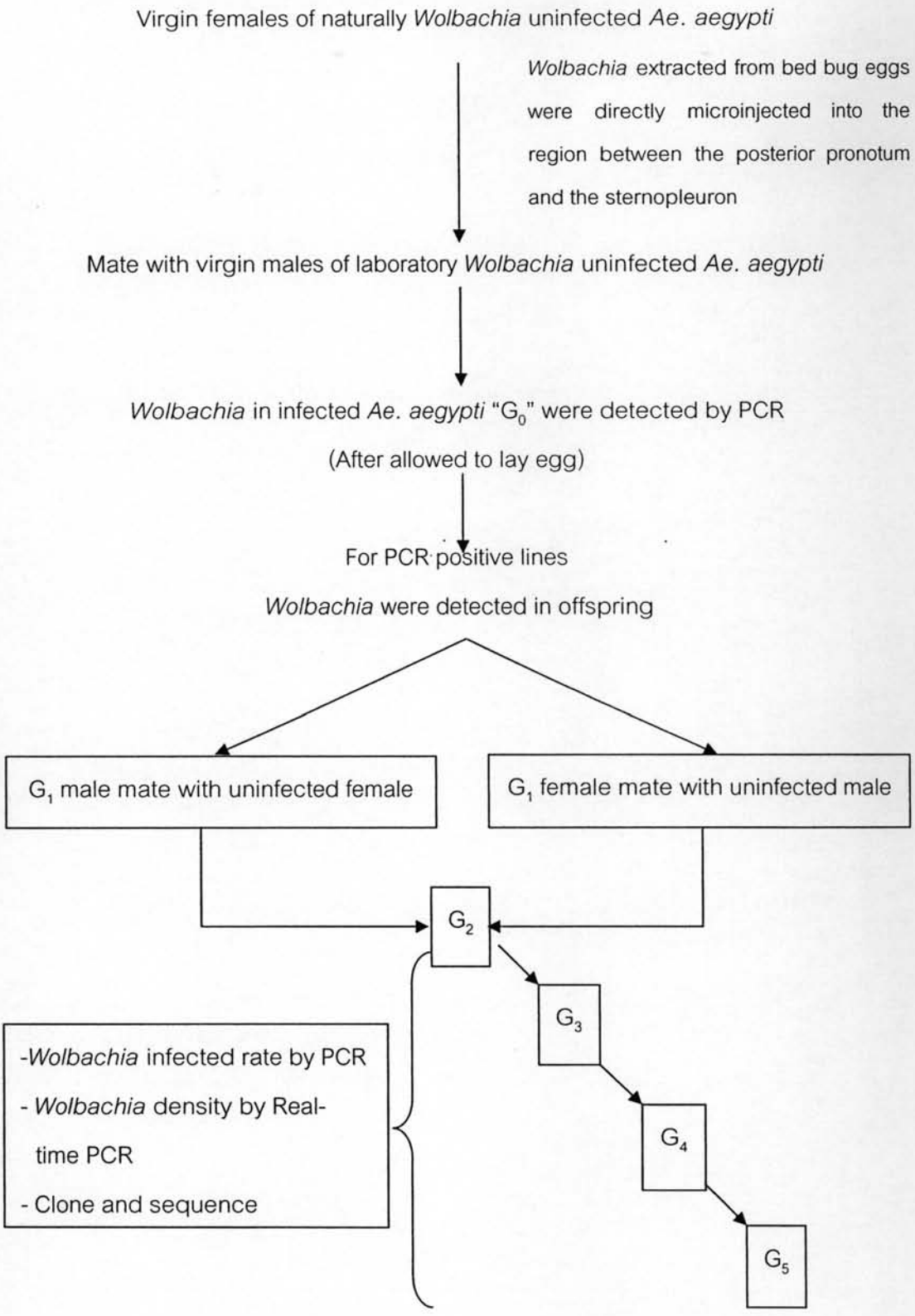
Wolbachia from *C. hemipterus* are able to transinfect, adapt to live in novel hosts and are transferred into offspring.

Artificial bed bug (*C. hemipterus*) *Wolbachia* could be induced CI expression wide spread rapidly into naturally uninfected populations.

Wolbachia density may be corresponded to rate of transinfection and capability to adapt to live in cellular of the novel hosts.

Wolbachia transinfection in *Ae. aegypti* may change the nucleotide base in the novel hosts.

5. Conceptual Framework



6. Key Words

Cytoplasmic incompatibility, *Aedes aegypti*, *Cimex hemipterus* and *Wolbachia*

7. Operational Definitions

Cytoplasmic incompatibility is a reproductive incompatibility between sperm and egg, such as mating between *Wolbachia* infected males with uninfected females or mating between infected individuals harboring different strains of *Wolbachia*. Result in a high rate of embryo mortality.

Ae. aegypti is one of the most important mosquito vectors-borne diseases to cause dengue fever epidemics in the tropical and sub-tropical regions of the world.

C. hemipterus is classified in the family Cimicidae and is obligatory hematophagous ectoparasites of birds, bats and humans. Bed bug is not commonly considered as vectors of disease but infestations can cause considerable distress to humans

Wolbachia is a group of gram negative intracellular bacterium and maternally inherited endosymbionts in the α-proteobacteria. They are inability to grow in cell free medium and can not be detected within infected gonadal cells. They are mostly found in reproductive tissues of arthropods and filarial nematodes. The first is found in *Culex pipiens*.

8. Obstacles

None

9. Expect Benefits and Application

9.1 Could be successfully to establish and transinfect *Wolbachia* F-supergroup from *C. hemipterus* into *Ae. aegypti*.

9.2 Information received and results of *Wolbachia* F-supergroup to induce CI in *Ae. aegypti* for reduce the mosquito populations.

9.3 Genetic modification strategies of arthropod vector to disease-blocking transgene for control disease.

9.4 Release transgene of *Ae. aegypti* for suppression, replacement and reproductive candidate with naturally populations. That means is genetic control.

9.5 This research has been application for the other research to clear about mechanisms, roles and functions of *Wolbachia* bacteria in the other novel hosts.