

RISK ASSESSMENT ON THE RELEASE OF WOLBACHIA- INFECTED AEDES AEGYPTI

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Foreword

The risk analysis for the research on the development of *Wolbachia*-infected *Aedes aegypti* was the initiative of the Directorate General of Research and Development Strengthening, the Ministry of Research, Technology and Higher Education (Ditjen Riset, Kemenristekdikti). The risk assessment was conducted as a follow-up to the mandate in the national stakeholders meeting on 12 February 2016 that the research on *Wolbachia*-infected *Ae. aegypti* conducted by the Eliminate Dengue Project Yogyakarta (EDP-Yogya) and supported by Tahija Foundation took safety and caution measures before conducting wide-scale release.

Together with the National Institute of Health Research and Development (Balitbangkes) of the Health Ministry, the Research and Development Directorate General of the Ministry of Research, Technology and Higher Education has established an independent core team of experts that consisted of five members who are responsible for conducting the risk assessment. The core team consisted of member experts from the universities, namely the Bogor Agricultural Institute (IPB), Airlangga University (Unair), Hasanuddin University (Unhas), and Gadjah Mada University (UGM) with various relevant backgrounds of expertise.

In addition to the core team of 5 expert members, the assessment also involved other 19 independent experts from universities, research institutes, non-government organisations, and the ministerial agencies to assess the risks associated with the research and development of *Wolbachia*-infected *Ae. aegypti* in a two-day workshop at the end of May 2016. After the main workshop, the core team of experts conducted a series of meetings to discuss the results of the risk analysis using the approved method which is Bayesian Belief Network (BBN) method.

This document presents the processes and results of the risk assessment on the development of *Wolbachia*-infected *Ae. Aegypti*, which has been submitted to the Research and Development DG, the Kemenristekdikti and Head of Balitbangkes and has also been presented in the national stakeholder meeting on 2 September 2016.

The risk assessment on the research on the development of *Wolbachia*-infected *Ae. Aegypti* has gone through a long transparent and accountable process. This document is expected to be used as a reference for the EDP Yogya researchers and stakeholders in overseeing the continuation of the research on the development of *Wolbachia*-infected *Ae. aegypti* to the next stages by ensuring the principles of safety and caution.

Jakarta, August 16, 2017



Dr. Muhammad Dimiyati
Director General of Strengthening for Research and Development
The Ministry of Research, Technology and Higher Education

LIST OF ABBREVIATIONS

Australian OGTR	Australian Office of The Gene Technology Regulator
Balitbangkes	Agency for Health Research and Development
BBN	Bayesian Belief Network
BMGF	Bill and Melinda Gates Foundation
CSIRO Australia	Commonwealth Scientific Industrial Research Organisation - Australia
CFR	Case Fatality Rate
CI	Cytoplasmic Incompatibility
CPT	Conditional Probability Table
DAG	Directed Acyclic Graph
DENV	Dengue virus
DHF	Dengue Haemorrhagic Fever
DNA	Deoxyribonucleic Acid
Depkes	Health Ministry
Ditjen P2P	Directorate General for Disease Prevention and Control
EDP	Eliminate Dengue Project
EIP	Extrinsic Incubation Period
ITIS	The Integrated Taxonomic Information System
IR	Incidence Rate
Kemenkes	Health Ministry
Kemenristekdikti	Research, Technology and Higher Education Ministry
MTA	Material Transfer Agreement
RA	Risk Assessment
RKD	Village Health Volunteer
SIS	Stakeholder Inquiry System
WHO	World Health Organization
YT	Yayasan Tahija

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CHAPTER 1

INTRODUCTION

Dengue virus is a pathogen that can cause the widespread explosion of Dengue Fever (DF) in many regions across Indonesia. A number of cosmopolitan insects such as *Aedes aegypti*, *Ae. albopictus* and other types of mosquitoes (WHO 1997, Pérez *et al.* 1998, CDC 2010) are the primary vectors of dengue virus. One of the manifestations of dengue virus is Dengue Haemorrhagic Fever (DHF), a fever that can lead to shock and event death in patients. The WHO classifies the severity of dengue virus infections into Dengue Fever and Dengue Haemorrhagic Fever (Grade I-IV) (WHO 2011). According to WHO (2011), dengue virus infections are characterised by different fever symptoms including dengue fever, dengue haemorrhagic fever that is accompanied by shock and other unusual manifestations such as encephalopathy and cardiomyopathy. The conditions of the environment and community's behaviour can also affect the development of the DHF disease that is transmitted by *Ae. aegypti* that will have an effect on the prevalence of DHF all year long. All age groups are vulnerable to the disease. This condition is common in tropical countries, including Indonesia.

Aedes aegypti was first reported to be found in Indonesia in 1968 in Jakarta and Surabaya. Karyanti (2009) reported that DHF was first diagnosed in 1968 in Jakarta and Surabaya and had since spread to all 33 provinces in Indonesia. The Centre for Epidemiology Data and Surveillance of the Health Ministry in *Jendela Epidemiologi 2010* bulletin indicated that 58 cases of DHF were reported that led to 24 cases of death. In 1968, there was a very high rate of mortality of 41.3% due to DHF. In 2013, a total of 112,511 cases of DHF were reported with an incidence rate 45.85. The number of DHF cases declined to 100,347 in 2014 with an incidence rate of 39.8 per 100,000 populations and a case fatality rate (CFR) of 0.92% (Figure 1.1). In 2014, the number of deaths from DHF was reported 107 people. At the end of 2014, the Directorate General of Disease Prevention and Control (Ditjen P2P) of the Health Ministry reported that 433 out of a total 508 districts (around 85.2%) in 34 provinces in Indonesia are endemic to DHF. At the same year, The Strategic Plan of the Health Ministry targeted to suppress the DHF cases as low as ≤ 51 per 100,000 populations and the target was successfully met by the government (Figure 1.2).

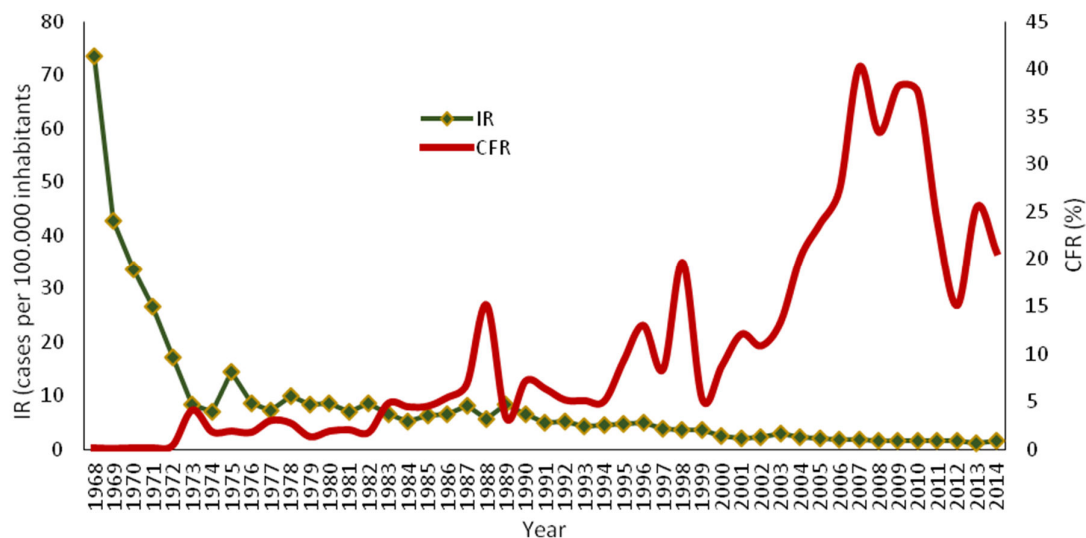


Figure 1.1 Development of the Incidence Rate (IR) and Case Fatality Rate (CFR) of Dengue Haemorrhagic Fever in Indonesia in 1968-2014 (updated by Ditjen P2P, 2015).

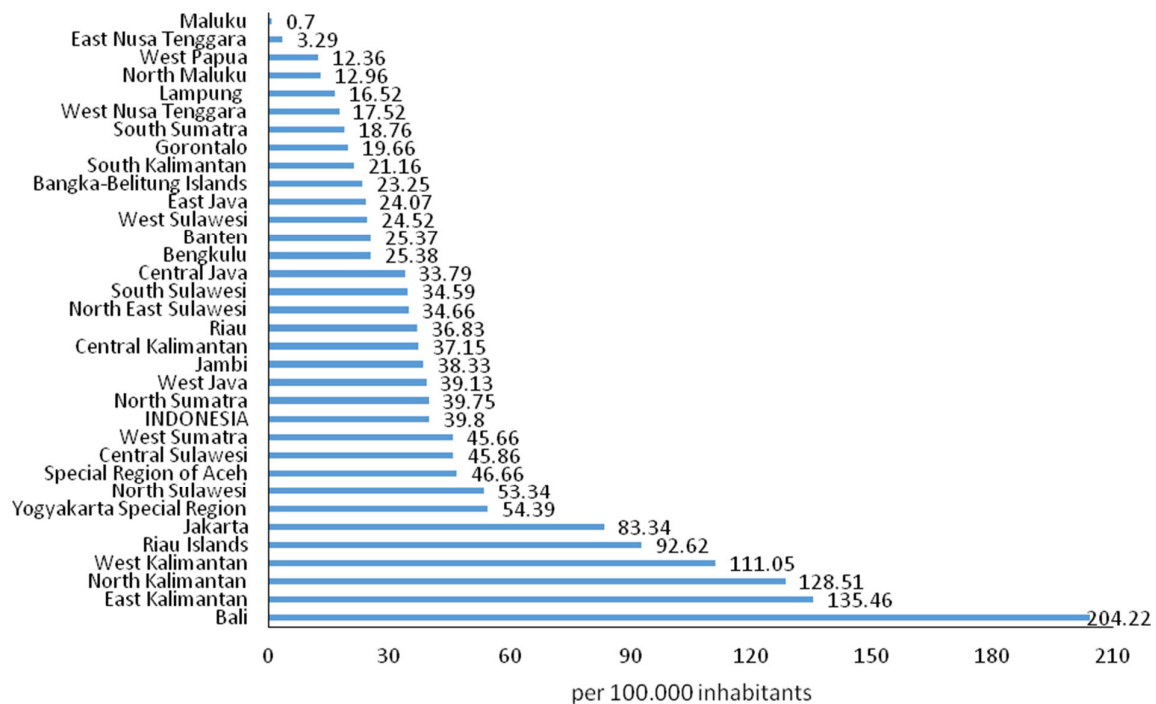


Figure 1.2 Incidence rate of dengue haemorrhagic fever cases in 2014 per 100,000 populations in each province in Indonesia (Ditjen P2P 2015).

In response to the recommendations by WHO (2004) that vector eradication had to be conducted to break the chain of DHF transmission, Indonesia has implemented a national “PSN 3M Plus Mosquito Breeding Site Eradication” strategy for the eradication of mosquitoes breeding sites by draining, covering and burying all water containers that can be used by mosquitoes for breeding sites. Other measures had also been conducted, including chemical fumigation (fogging), the use of mosquito repellents or spray insecticides, and sprinkling larvacides (abate) powder in water containers. Although mosquito eradication efforts have been conducted continuously, there is still a relatively high rate of DHF cases. As a result, a new technique for controlling DHF in Indonesia through the introduction of *Wolbachia*-infected mosquitoes were taken into consideration (WHO 2016). The World Health Organization Vector Control Advisory Group (WHO VCAG) has recommended the carefully planned pilot release accompanied by rigorous monitoring and evaluation that establishes entomological capacity to support of field operations. In addition, WHO also indicated that a study using Randomized Control Trial (RCT) design using epidemiological outcomes should be carried out to provide the evidence for the use of *Wolbachia* technology in the program.

The technique for controlling mosquitoes using *Wolbachia* is a new technique introduced by the **Eliminate Dengue Program Global (EDP Global)**. EDP Global is a non-profit organisation that researches into technology in the use of *Wolbachia* in controlling dengue transmission in Australia, Vietnam, Colombia, Indonesia, and Brazil. EDP Global is headquartered in Australia. The Eliminate Dengue Program has developed the natural method to reduce the transmission of dengue virus using *Wolbachia* bacteria. *Wolbachia* is known to have the ability to suppress replication of dengue virus in *Ae. aegypti* that it is expected to be able to reduce the ability of the mosquito to transmit dengue virus.

In Indonesia, the new approach using *Wolbachia* has been pioneered by the Centre for Tropical Medicine, the Faculty of Medicine, Gadjah Mada University since 2011. The use of the new technology has become all the reason to conduct a risk assessment.

An **Indonesian Risk Assessment (RA)** team was established by the Kemenristekdikti to identify future potential risks that may occur over a time period of 30 years associated with the release of *Wolbachia*-infected *Ae. aegypti*. The team conducted a risk assessment on the risks associated with the release of *Wolbachia*-infected *Ae. aegypti* using a methodology that was developed by the Commonwealth Scientific Industrial Research Organisation (CSIRO), Australia (Murray *et al.* 2016). The risk assessment team consisted of a core team with background expertise in medical entomology, biological evolution and medicine. In addition to the core team, a number of experts in different areas were also selected to participate in the discussions on the risk assessment. The expert team is composed of microbiology, medical entomology, parasitology, economy and social culture, public health, management of dengue diseases and bioethics experts (Figure 1.3).

A number of meetings and workshops were conducted to elicit opinions and evidence for the analysis of risks associated with the release of *Wolbachia*-infected mosquitoes. The Risk Assessment consisted of identification of different hazards that may have impacts on humans and the environment. Based on the results of discussion among experts involved in the meetings and workshops, four main categories of hazards were identified, as follows: **Ecological, Economic and Socio-Cultural, Efficacy in Mosquito Management, and Public Health**

The **Bayesian Belief Networks (BBN)** was used in developing the **Risk Assessment Framework**. Scoring was done to assess the estimate of **consequence** and **likelihood** of hazard occurrences. The estimate is aimed at establishing the likelihood of the hazards to occur and to determine the risk for **end-point**. The product of consequence x likelihood is the **risk estimation matrix**, which is the final tally of the risk analysis. Based on the risk matrix, risk estimates associated with the release of *Wolbachia*-infected *Ae. aegypti* were established on the following scales: almost negligible or very low risk, low, moderate, high and very high. From the results of the risk estimates, conclusions and recommendations could be drawn and developed for further use by the stakeholders.





Figure 1.3 The Indonesian Risk Assessment team, consisting of Prof. Dr. Ir. Damayanti Buchori, MSc (Chair); Prof. Dr. drh. Upik Kesumawati Hadi, MS; Prof. dr. Hari Kusnanto Joseph, SU, DrPH; Prof. Dr. dr. Aryati, MS, SpPK (K) and Prof. dr. Irawan Yusuf, MSc, PhD.

CHAPTER 2

BIOECOLOGY OF *Aedes aegypti* AND *Wolbachia*

2.1 *Aedes aegypti* (L.)

2.1.1 Taxonomy and Scientific Nomenclature

Scientific name	: <i>Aedes</i> (Stegomyia) <i>aegypti</i> (Linnaeus) (Insecta: Diptera: Culicidae)
Common name(s)	: <i>yellow fever mosquito</i> , <i>stegomyie</i> (Perancis), <i>yellow fever mosquito</i> (Inggris), nyamuk demam berdarah (Indonesia).
Synonym(s)	: <i>Culex aegypti</i> Linnaeus 1762 <i>Culex excitans</i> Walker 1848 <i>Culex taeniatatus</i> Weidemann 1828 <i>Aedes aegypti</i> Mattingly, Stone, and Knight 1962
Taxonomic Classification :	
Kingdom	<u>Animalia</u> – Animal, animaux, animals
Subkingdom	<u>Bilateria</u>
Infrakingdom	<u>Protostomia</u>
Superphylum	<u>Ecdysozoa</u>
Phylum	<u>Arthropod</u> – Artrópode, arthropodes, arthropods
Subphylum	<u>Hexapod</u> – hexapods
Class	<u>Insecta</u> – insects, hexapoda, inseto, insectes
Subclass	<u>Pterygota</u> – <i>insects ailés</i> , <i>winged insects</i>
Infraclass	<u>Neoptera</u> – <i>modern</i> , <i>wing-folding insects</i>
Superorder	<u>Holometabola</u>
Order	<u>Diptera</u> – <i>mosca</i> , <i>mosquito</i> , <i>gnats</i> , <i>mosquitoes</i> , <i>true flies</i>
Suborder	<u>Nematocera</u> – <i>long-horned flies</i>
Infraorder	<u>Culicomorpha</u>
Family	<u>Culicidae</u> – <i>mosquitoes</i> , <i>maringouins</i> , <i>moustiques</i>
Subfamily	<u>Culicinae</u>
Tribe	<u>Culicini</u>
Genus	<u>Aedes</u> Meigen 1818
Species	<u><i>Aedes aegypti</i></u> (Linnaeus 1762) – <i>yellow fever mosquito</i> , <i>stégomyie</i> , <i>yellowfever mosquito</i>

Source: ITIS (2016), the Integrated Taxonomic Information System (<http://www.itis.gov/>) and ICZN (2016) International Commission on Zoological Nomenclature (<http://www.iczn/index.jsp>).

There are currently 3,000 species of mosquitoes, that are grouped into 39 genus and 135 subgenus. Genus *Aedes* (Diptera: Culicidae) has at least 700 that are classified into a number of subgenus including *Aedes* and *Stegomyia* (Mousson *et al.* 2005). In Indonesia, 450 mosquito species have been identified and described, including 80 *Aedes* species (O'Connor and Sopa 1981). Two species of *Aedes*, i.e *Aedes aegypti* and *Ae. albopictus* are respectively primary and secondary vectors of dengue diseases in Indonesia.

2.1.2 Description

Aedes aegypti is a mosquito of 4-7 mm in size. The morphology of *Ae. aegypti* resembles *Ae. Albopictus*. *Ae. albopictus* is the second primary vector of dengue virus in Indonesia. Both species are distinguished by the size and patterns of their thorax. The adults of *Ae. aegypti* have white scales on the dorsal surface of the thorax that form the shape of a violin or lyre while adult *Ae. Albopictus* have a white stripe down the middle of the top of the thorax (Figure 2.1). The tarsal segments of the hind legs have white basal bands that form what appears to be short stripes. The abdomen is generally dark brown to black, but also may possess some white scales (Carpenter and LaCasse 1955; Christophers 1960).



Figure 2.1 *Aedes aegypti* (left) with a U shape on the dorsal surface of the thorax and *Ae. albopictus* with white stripe on the dorsal surface of the thorax (Lounibos and O'Meara 2009).

Females are larger than males, and can be distinguished by small palps tipped with silver or white scales. Males have plumose antennae (with lots of short hairs) while females have plumose antennae with sparse short hairs (pilose antennae). Male mouthparts are modified for feeding on nectars and female mouthparts are modified for blood feeding. The proboscis of both sexes is dark, and the clypeus (segment above the proboscis) has two clusters of white scales. The tip of the abdomen of both species are almost similar, which is the characteristic of all *Aedes* species (Cutwa-Francis and O'Meara 2007).

2.1.3 Biology

Aedes aegypti is a holometabolous insect, meaning that it goes through a complete metamorphosis from egg through four larval stages, pupation, and imago or adult stage. The eggs will hatch into larvae within 1-2 days at room temperature and 70-80% humidity. The larval stages take 5-6 days while the pupal stage lasts for 2-3 days. In Indonesia, the life cycle of *Ae. aegypti* lasts 8-11 days on room temperature (Hadi *et al.* 2006). The length of the larval stadium is dependent on

temperature and nutritional content of the food. The growth of the larval stages can affect the fitness of adult mosquitoes in producing offspring. During the pupal stage (resting phase), *Ae. aegypti* do not feed and the success of this stage is dependant on the energy obtained during the larval stages (Hadi *et al.* 2006).

Male adult *Ae. aegypti* have a life span of 14 days while female adult *Ae. aegypti* can live for several months. One female mosquito can lay more than 600 eggs (Hadi *et al.* 2006). In Argentina, fecundity ranges from 47.1 ± 14.2 to 307.4 ± 86.4 eggs per female (Tejerina *et al.* 2009). *Ae. aegypti* eggs in Argentina are resistant to desiccation and can survive for a few months to more than one year (Christophers 1960). The eggs lay firmly on bathroom walls or tub, jars, cans and other containers that hold water and form stagnant water puddles during the rainy season. During the dry season, the containers will dry but the eggs survive until the time of the rainy season or when the containers filled with water. During the beginning of the rainy season, the eggs will hatch into as larvae. The ability of the eggs to survive in desiccation for a long time is the key factor that allows eggs to be easily spread to new locations.

The breeding of *Aedes aegypti* is tied exclusively to artificial containers holding water such as bath tubs, water tanks, jars, discarded tyres, and other water containers in the presence of human habitation (Montgomery *et al.* 2004; Hadi and Koesharto 2006, Zahara *et al.* 2015). Male adult *Ae. aegypti* feed on nectars of plants and female adult feed primarily on human blood (Scott *et al.* 2000; Harrington *et al.* 2001; Hadi 2016) and other blood meals from other hosts that available during the oviposition cycle (Scott *et al.* 1993; Michael *et al.* 2001; Hadi 2016). At certain condition, female *Ae. aegypti* also feed on the blood of birds and livestock animals (Tandon and Ray 2000).

Female *Ae. aegypti* predominantly show endophagic and endophilic behaviours where they feed and rest indoor (Surtees 1967). The female of *Ae. aegypti* are diurnal; they tend to feed on blood during the day time in the morning around 08.00-09.00 and the evening around 16.00-17.00 (Christophers 1960; Gubler and Meltzer 1999; Hadi and Koesharto 2006). Oda *et al.* (1983) reported that in Jakarta, mosquito attacks (biting activity) usually last the whole day long from sunrise to sunset. Novelani (2007) reported that in Utan Kayu, East Jakarta, *Ae. aegypti* show endophagic pattern of behaviour where they are active from 08.00-18.00 while *Ae. albopictus* (the second main vector of dengue diseases) show exophagic behaviour – biting activity occurs in outdoor – and are active during 08.00-10.00 and 16.00-06.00. Riwu (2011) reported that *Ae. aegypti* in Pasir Kuda, Bogor also show active endophagic behaviour during 11.00-12.00 (6.81 mosquitoes/human/hour) and during 14.00 to 15.00 (6.5 mosquitoes /human/hour), while *Ae. albopictus* tend to be exophagic during 07.00-08.00 (1.13 mosquitoes /human/hour) and 15.00-16.00 (1.31 mosquitoes /human/hour). Riwu (2011) added *Ae. aegypti* tend to rest indoor (endophilic), while *Ae. albopictus* outdoor (exophilic).

Female mosquitoes begin to produce eggs as soon as they feed on enough blood meals. The number of eggs produced depend on the amount of the blood meals. Adult female can produce up to 5 batches of eggs during their lifetime. Less blood meals produce fewer eggs (Nelson 1986). Eggs are laid on water surfaces such as tree holes and water containers, and are laid singly, rather than in a mass. Not all the eggs are laid at once, but can be spread out over hours or days, depending on the availability of suitable substrates (Clements 1999). Most often, eggs are placed at varying distances above the water surface, and a female will not lay the entire clutch at a single site, but rather spread out the eggs over two or more sites (Foster and Walker 2002).

2.1.4 Distribution and Dispersal

Aedes aegypti are commonly found in tropical areas and can breed the whole year round. In most sub-tropical areas, *Ae. aegypti* are only found during summer time (Figure 2.2). Figure 2.3 indicates the global evidence consensus, risks and burden of dengue in 2010 (Bhatt *et al.* 2013). *Ae. aegypti* are suspected to originate from Africa but is now distributed globally in tropical and subtropical regions

and in some of temperate climate regions through global trade and shipment (Powell and Tabachnick 2013).

The global distribution of *Ae. aegypti* is commonly assisted by mass human migrations. The first process of distribution was suspected to be associated with the migration to the New World through slave trade between the 15th and the 19th century and secondly as a result of the migration to Asia during the 18th and the 19th century. Wider distribution to various countries across the world occurred during the third migration after the World War II (Mousson *et al.* 2005).

Aedes albopictus originate from Asia and have gone through almost similar processes of distribution with *Ae. aegypti* through global mass human migration in regions with tropical, sub-tropical and temperate climates, especially through international trade of discarded tyres (Reiter and Sprenger 1987, Hawley 1988). *Ae. albopictus* have been adapting to survive in wider ranges of temperature and in extreme colder temperature that they manage to survive in regions with temperate climate. *Ae. albopictus* live around human residential areas, a little bit different with *Ae. aegypti* that live in human habitation.

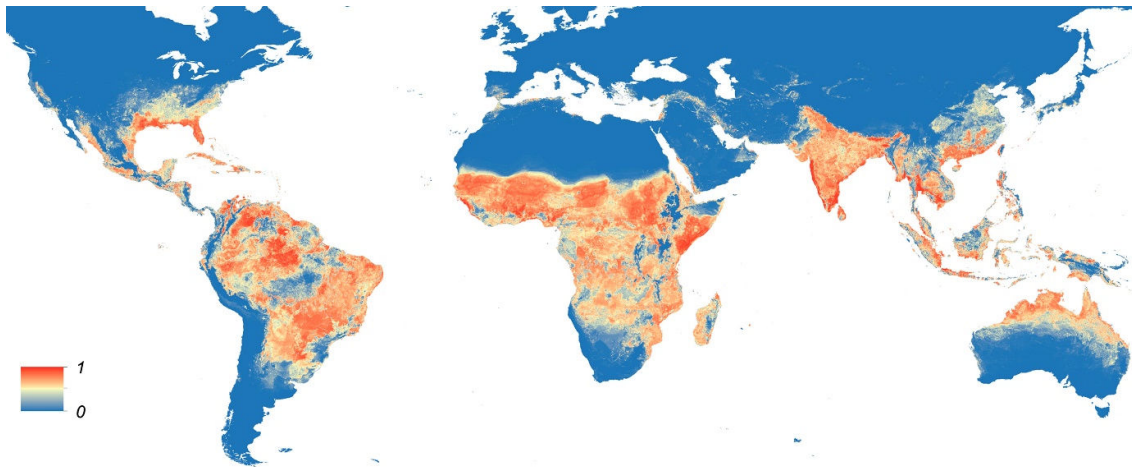


Figure 2.2 Global distribution map of *Ae. Aegypti*. The map depicts the probability of occurrences (from 0 blue to 1 red) (Kraemer *et al.* 2015).

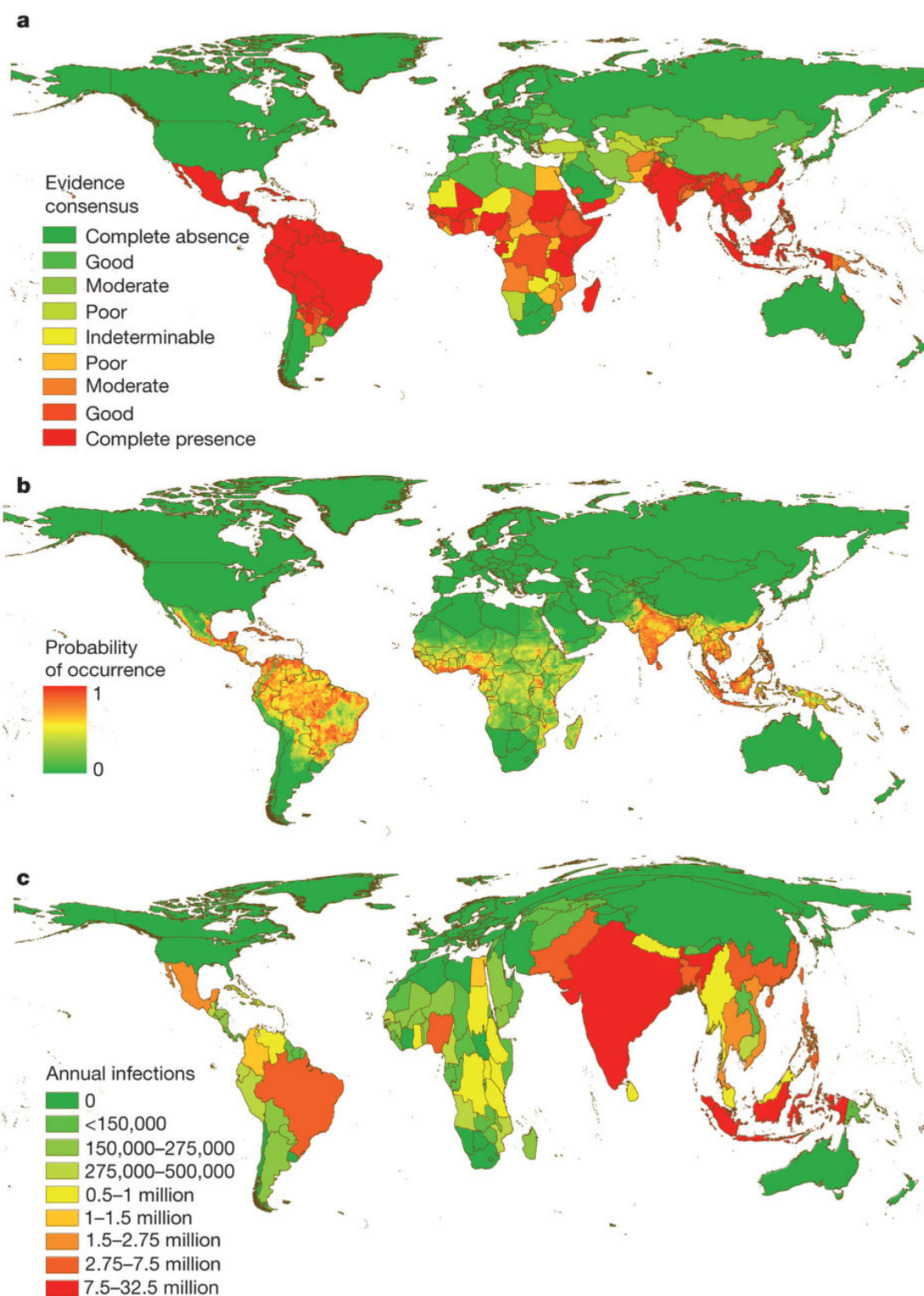


Figure 2.3 Global evidence of consensus, risks and burden of dengue in 2010 (Bhatt *et al.* 2013).

In Indonesia, *Ae. aegypti* and *Ae. albopictus* are the primary vectors of dengue diseases that are prevalent in all regions in Indonesia, except in regions at more than 1,000 metres above the sea level (Hadi and Koesharto 2006). Takahashi *et al.* (2005) divided the dispersal of *Ae. aegypti* into three types. The first type by females flight capacity in searching for blood meals or laying eggs. Such mode result in very limited spread. The second type is dispersal assisted by wind current while the third one is the mode of longer distance dispersal that is the result of anthropogenic activities, particularly by transportation systems that help mosquitoes to circumvent natural barriers and to cover thousands of kilometres distance from their original sites during their lives (Harrington *et al.* 2005, Reiter 2007).

Female *Ae. aegypti* spend their lifetime in or around the houses where they emerge as adult. Female mosquito usually has a range of flight of around 400 metres. It means that humans have more important roles than mosquitoes in the distribution of viruses between communities and locations. Reiter (2007) conduct an evaluation of the available data on the mark and release of *Ae. aegypti* and concluded that initial estimates of daily flight capability ranging from 25-30 m underestimated the dispersal ability of mosquitoes, as mature adults could cover over 11 km distance. A key factor in dispersal is the 'need' to disperse and so may be influenced by the size of available feeding hosts and oviposition (egg laying) sites such as water containers. Absence of one of those factors could trigger longer distance dispersal. In Yogyakarta, particularly, the populations of *Ae. aegypti* tend to be stable in all seasons. Genetic variations are very much influenced by the conditions of the habitat. Habitat where residential homes are bordered with natural barriers such as vegetation have greater genetic variations compared to the one where residential homes are attached with each other (Tantowijoyo *et al.* 2015).

2.1.5 Medical Importance

When *Ae. aegypti* bites human, it will leave itchy rash and skin irritation on the bitten spot. Medically, the bites of *Ae. aegypti* are harmless; they only leave uncomfortable feeling when doing outdoor activities. However, the bites do bring harm because *Ae. aegypti* is the primary vector of several arboviruses (viruses transmitted by arthropods) such Chikungunya, yellow fever, Zika virus and most particular dengue haemorrhagic fever (DHF) (Pialoux *et al.* 2007; Hadi 2016). Dengue is a positive sense single stranded RNA [(+)ssRNA] virus of approximately 10-11 kb in size in the genus *Flavivirus* (Henchal & Putnak 1990; Russell & Dwyer 2000). Molecular studies have shown that *Flavivirus* arboviruses have undergone an explosive radiation in the last 200 years, attributed to the worldwide intermixing of hosts, vectors and virus as a result of ever increasing and dispersing human populations (Zanotto *et al.* 1996).

DHF was first recorded to occur in Southeast Asia during the 1950s. However, it was not until 1975 that DHF becomes the leading cause of death among children in various countries in Asia (Bang and Shah 1986). Globally, the prevalence and endemicity of the disease has increased drastically in more than 100 countries in Africa, America, Middle East, Southeast Asia and West Pacific. Southeast Asia and West Pacific countries are countries with most cases of DHF. Before the 1970s, only 9 countries had experienced DHF epidemic. The number has multiplied fourfold in 1995. In 1997, WHO declared DHF as the most leading viral disease that can cause harm to human (WHO 2004). Data from WHO (2009) indicated that at least 2.5 billion of the world population lives in areas endemic to DHF as a result of the increasing geographical distribution of mosquitoes as the vector and dengue virus during the last 25 years. Since 1955 until 2007, and according to the update data of WHO for 2004-2010, there has been an increase in the number of areas endemic to DHF, particularly urban areas in tropical countries (Figure 2.4). Indonesia comes second in terms of highest DHF endemicity with 129,435 cases after Brazil (447,466 cases), and Indonesia has the highest endemicity among ASEAN countries with the highest number of DFH cases.

Latest global estimate by Bhatt *et al.* (2013) indicated a figure of three times higher than the dengue burden estimate by WHO, that is 390 million cases of dengue infections per year, of which 96 million cases had clinical manifestation with various levels of severity.

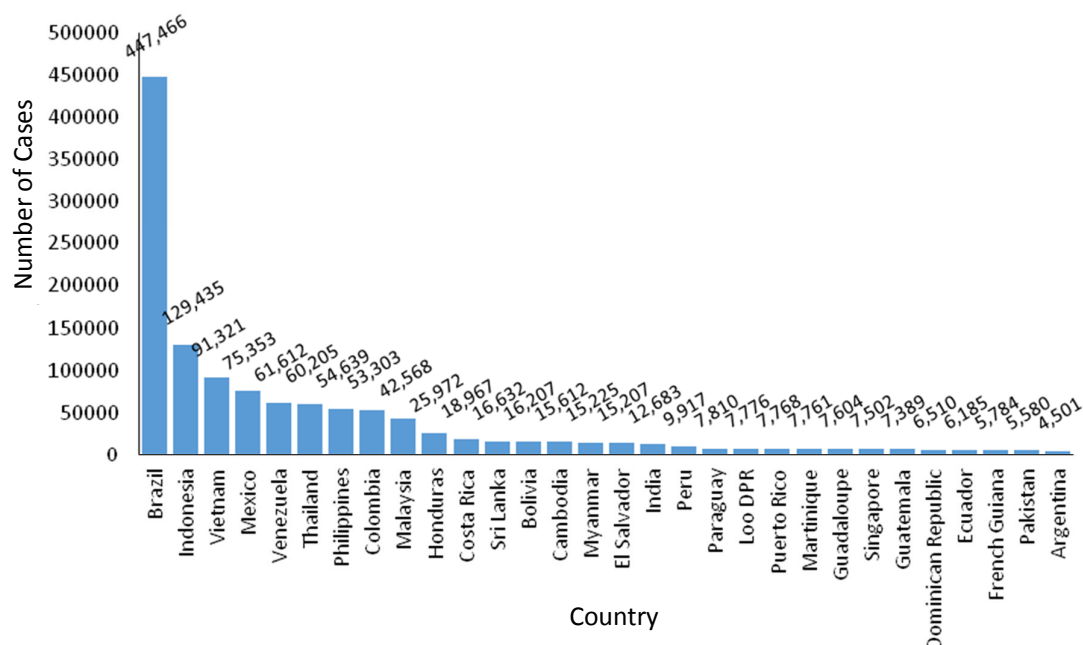


Figure 2.4 Average number of Dengue cases in 30 countries with the highest endemicity, as report to WHO 2004-2010 (WH 2012).

DHF was first recognised in Indonesia in 1968 in Jakarta and Surabaya (Bang and Shah 1986). A dengue haemorrhagic fever epidemic in outside of Java island was first reported in West Sumatra and Lampung in 1972 and later spread widely to different regions in Indonesia. The Ministry of Health of the Republic of Indonesia (Kemenkes RI 2014) reported that in 2013, a total 90,245 cases of DHF were reported with 816 deaths (IR of 37.27 per 100,000 population and CFR of 0.90%). The highest number of deaths due to DHF in 2013 was found in West Java (167 cases), East Java (114 cases) and Central Java (108 cases), while deaths was lowest in Jakarta (4 cases) (Figure 2.5). This is an indication that Jakarta has a relatively good system for DHF monitoring and management compared with other regions.

Dengue shows a range of clinical symptoms widely known as the 'break bone fever' syndrome, which is accompanied by headaches, muscle pain, nausea etc (Gubler 1998). Until now dengue viruses (DENV) are believed to be caused by four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) and it is not uncommon to have several serotypes circulating simultaneously in a region. In October 2013, the fifth serotype DENV-5 was successfully isolated but further research need to be undertaken (Mustafa *et al.* 2015). Exposure to one serotype provides lifelong immunity, but not cross immunity to other serotypes. Despite immunity to one serotype, exposure to other three serotypes can increase the risk of contracting the more serious Dengue Haemorrhagic Fever (DHF). This has been the cause of over 70,000 deaths since the 1950s worldwide (Deen 2004). The Indonesian Ministry of Health (2014) has reported that DENV-3 serotype is the most commonly found dengue virus in most of the regions in Indonesia.

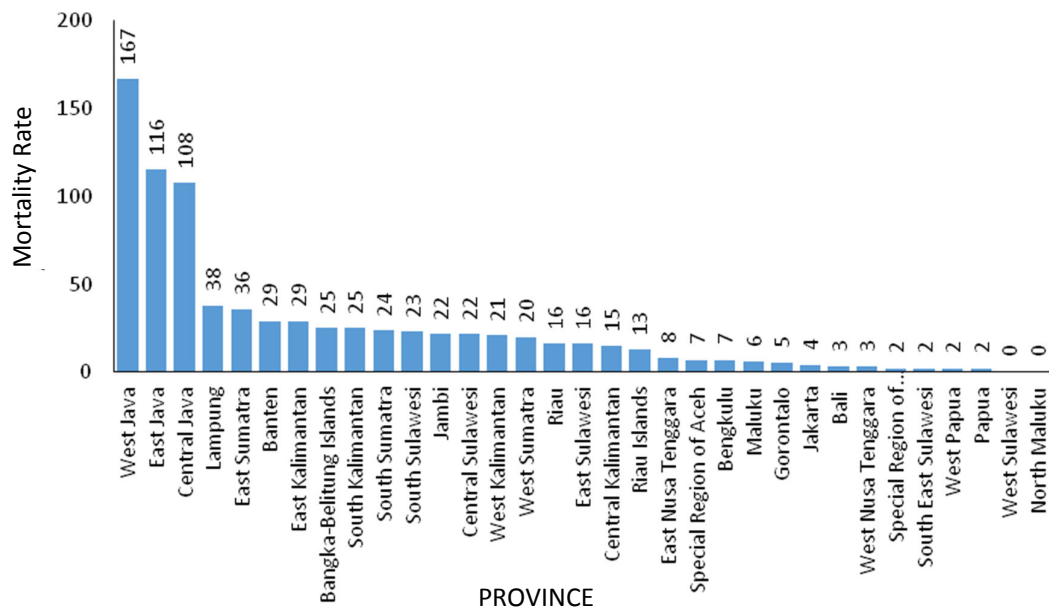


Figure 2.5 Number of DHF deaths per province in Indonesia in 2013 (Kemenkes RI 2014).

The intrinsic incubation period of dengue virus in human lasts 4-7 days, approximately 3-14 days after the virus transmitted through the bites of infected female mosquitoes. The fever stage lasts on average in five days. Fever happens due to blood feeding by vector and the virus entering the peripheral blood supplies. The extrinsic incubation period (EIP) is the time required for the virus to replicate in the mosquito before it can be transmitted. The extrinsic incubation period for dengue virus in *Ae. aegypti* is 8-12 days (Gubler and Meltzer 1999; Brownstein *et al.* 2003). This lag between the uptake and virus transmission explains why adult female mosquitoes play the important role in virus transmission. The virus remains in the infected mosquitoes for the rest of their life. Therefore, *Ae. aegypti* that has been infected by dengue virus will become the vector for transmitting virus for the rest of its life. Infected mosquitoes can transmit dengue virus vertically to their offspring via the eggs (Joshi *et al.* 1996) although controversy still remains on the importance of transovarial transmission.

According to Esteva and Vargas (2000), DHF is only endemic to tropical areas where a combination of suitable climate and weather allows mosquitoes to breed all year round. DHF is not endemic to Australia and temperate and subtropical regions but they will always be present due to continued reintroduction by infected travellers (Gould and Solomon 2008).

2.1.6 Dengue Vector Control

The prevention or elimination of dengue virus transmission depends greatly on vector control and prevention of contacts between mosquitoes and humans. Surveillance on the distribution and development of mosquito population in a region is a main component in integrated vector management program. Mosquito surveillance aims at measuring the risks to human by determining the presence and abundance of vector in one particular area. In Indonesia, a national dengue vector management program has been ongoing since 1982 but many cases of DHF continue to occur and the rate has been increasing on annual basis. The most popular government dengue vector management program is the national Breeding Site Eradication (Pemberantasan Sarang Nyamuk/PSN) program for the elimination of mosquito breeding sites through the “3M plus” action of covering, draining, and burying discarded water containers. The program promoted by the Health Ministry has recommended the participation of the local community in conducting regular entomological survey in their respective

places of residence. In some villages in Indonesia, Village Health Volunteers (RKD) are already in place who are trained to conduct the survey. The volunteers periodically conduct entomological surveys in the villages under their assistance. Other programs include the improving of water supplies, mosquito biological control using natural enemies such as predator fish, insecticides (spraying or fogging and larval control), as well as health education and community empowerment (Kemenkes RI 2014).

2.2 WOLBACHIA

2.2.1 *Wolbachia*

Wolbachia are gram negative bacteria that cause intracellular infections in invertebrates. *Wolbachia* belong to the order *Rickettsiales* and are classified as strains of one species (*Wolbachia pipientis*) (Perlman *et al.* 2006). *Rickettsiales* has a more dynamic genome with more repeats and labile genetic components than found in other eukaryote-inhibiting bacteria (Wernegreen 2005), including a unique bacteriophage playing the role as WO phage (Sanogo *et al.* 2005) that is associated with *Cytoplasmic Incompatibility* (CI) (Bordenstein and Reznikoff 2005).

Wolbachia is known to have its effect in reducing the fitness and lifespan of *Drosophila melanogaster* through prolific replication that causes damage in host tissue (Min and Benzer 1997). In addition to reducing lifespan, *Wolbachia*, particularly the wMel strain, also causes the 'bendy proboscis' phenomenon in ageing female *Ae. aegypti*. With bendy proboscis, adult female cannot penetrate into human skin to feed on blood (Turley *et al.* 2009).

There are two *Wolbachia* strains originating from *Drosophila melanogaster* that are introduced to *Aedes aegypti*; wMel and wMelPop. Both strains are easily found in natural populations of *D. melanogaster* and in *D. melanogaster* that has been cultured in the laboratory for a considerable time (Bourtzis *et al.* 1994, Hoffman *et al.* 1998). wMel and wMelPop suppress the transmission of dengue virus. *Wolbachia* has genomes that range between 1 to 1.6 Mb in size. The genome sizes of wMel, wMelPop and wMelCS are 1.36 Mb for each strain, while other strains have smaller sizes such as wRI 1.66 Mb, wBma 1.1 Mb, and wDim 0.95 Mb (Sun *et al.* 2001).

The major genetic difference between wMelPop and wMel is a single genomic inversion (Sun *et al.* 2003). wMel was thought to contain large amounts of repeated DNA mobile genetic elements (Wu *et al.* 2004) although subsequently it was found that wMel has a smaller genome and less mobile elements than in wPip (Klasson *et al.* 2008). Both mtDNA and *Wolbachia* are maternally inherited that *Wolbachia* have been associated with decreases in mtDNA diversity (Hale and Hoffmann 1990; Hurst and Jiggins 2005). Riegler *et al.* (2005) have found evidence that there was a higher frequency of wMel in *D. melanogaster* that carried a particular mtDNA haplotype. Turelli *et al.* (1992) observed *Wolbachia* infection of *D. melanogaster* in California and found that all infected flies had the same mtDNA haplotype.

Wolbachia has been observed in a wide range of invertebrates including crabs, mites and filarial nematodes (Sun *et al.* 2003) and it generally behaves as parasites in arthropod hosts and as mutualists in nematodes (Mercot and Poinot 2009). There is some controversy as to whether the *Wolbachia* that infect filarial nematodes should be classified as a separate species from the one that infect insects. Both *Wolbachia* have different roots within the *Wolbachia* clades. Besides that, they also have very distinct biology (Pfarr *et al.* 2007). The presence of *Wolbachia* in hosts can be detected by PCR using primers specific to *Wolbachia* such as the *Wolbachia* surface protein (*wsp*) gene (Dobson *et al.*, 1999) or *ftsZ* gene (Lo *et al.*, 2002).

It is estimated that around 75% of arthropod species (Stevens *et al.* 2001) and 20% of all insect species contain *Wolbachia* (Cook and Butcher 1999). However, this is likely an underestimate due to low

prevalence of infections, inadequate sampling and false negatives PCR resulting from PCR primer sets that could not amplify all *Wolbachia* (Weinert *et al.* 2007). *Aedes aegypti* is not known to naturally harbour *Wolbachia* (Ruang-areerate and Kittaypong 2006).

Wolbachia are maternally transmitted and infect host reproductive tissues. The bacteria then manipulate the reproductive cycle of the hosts to increase their own transmission (Werren 1997). Reproductive strategies that are associated with *Wolbachia* infection include parthenogenesis, male killing or feminization, sex ratio distortions (Cook and Butcher 1999; Hurst *et al.* 2002) and cytoplasmic incompatibility (CI) (McGraw and O'Neill 2004). Although until now it has not yet been resolved exactly how CI is achieved, cytoplasmic incompatibility is thought to have the potential to control transmission of viruses/pathogens by disease vector arthropod. CI provides a drive mechanism for *Wolbachia* to be able to invade a target host species and block viruses (Poinsot *et al.* 2003; Kent and Norris 2005).

CI causes asymmetric mating so that *Wolbachia*-infected adult females can mate successfully with either infected or uninfected males and produce offspring while mating between *Wolbachia*-uninfected females with infected males produce sterile offspring. Multiple *Wolbachia* strains may be found within a host species, leading to super-infections and bi-directional incompatibility within populations (Hoffmann and Turelli 1988).

CI potentially allows a small number of *Wolbachia* propagules to enter an uninfected population of mosquitoes. This has been successfully observed both in the field (Hoffmann *et al.* 1986; Turelli and Hoffmann 1991) and in the laboratory, e.g. after Xi *et al.* (2005) successfully introduced the wAlbB strain (the *Wolbachia* strain from *Aedes albopictus*) into a caged *Aedes aegypti* population. The presence of *Wolbachia* strain can be maintained within seven generations. Brownstein *et al.* (2003) used a model to show that although with low initial frequencies of *Wolbachia* infected *Ae. aegypti* (0.2- 0.4), the mosquitoes could drive into and survive in a population and substantially reduce dengue virus transmission. However, this was limited by the rate of CI achieved and any form of reduction on host fecundity.

Wolbachia can induce phenotypes that can lead to a range of beneficial, neutral or pathogenic impact on hosts (Stouthamer *et al.* 1999; Weeks *et al.* 2002) including life extension due to limited availability of suitable diet (Mair *et al.* 2005) or shorter longevity (Min and Benzer 1997), increased immune response to filarial nematodes (Kambris *et al.* 2009), increased fecundity (Vavre *et al.* 1999) or reduced fecundity (Hoffmann *et al.* 1990), reduced ability to disperse (Silva *et al.* 2000) and reduced ability for survival and locomotor performance among adults (Fleury *et al.* 2000).

Wolbachia is also involved in establishing resistance to RNA viruses in their hosts by delaying accumulation of the virus. Through RNA virus infection treatment to *Wolbachia*-infected *D. melanogaster* and *Wolbachia*-uninfected *D. melanogaster*, Teixeira *et al.* (2008) and Hedges *et al.* (2008) demonstrated that *Wolbachia* infected *D. melanogaster* lived significantly longer than the uninfected flies. However, this resistance did not apply to a DNA virus. In addition, it is known that *Wolbachia* can interfere a range of pathogens infecting *Ae. aegypti* such as filarial nematodes, bacteria, dengue and Chikungunya viruses as well as (Kambris *et al.* 2009; Moreira *et al.* 2009).

The close association between *Wolbachia* and host reproductive tissues is expected to increase the possibility of horizontal gene transfer events. Comparison of arthropods genomes and *Wolbachia* indicate horizontal gene transfers often occurred, but the majority of exchanged material is non-functional (Woolfit *et al.* 2009). Dunning-Hotopp *et al.* (2007) found evidence that transfer of *Wolbachia* in the genomes occurred in four insect species and four nematode species. Klasson *et al.* (2009) identified a functional gene in *Ae. aegypti* that was found to be associated with a *Wolbachia*

horizontal gene transfer event. Woolfit *et al.* (2009) found that the unique genes coding for salivary gland surface (SGS) proteins for mosquitoes (including *Ae. aegypti*) had putative homologs in *Wolbachia*. Such genetic evidence suggests that transfer can occur from the eukaryote to the bacteria rather than the other way around. In addition to genetic exchange, *Wolbachia* interact directly with host genomes as indicated by the results of the research by Xi *et al.* (2008) indicating that *Wolbachia* activating genes in *Drosophila* hosts that facilitated *Wolbachia* movement into host reproductive tissues.

2.2.2 Modification Methods

Wolbachia can be routinely cultured in insect cell lines (O'Neill *et al.* 1997; Jin *et al.* 2009). However, transfer of *Wolbachia* to novel hosts by microinjection can be technically challenging due to its unpredictable success (McMeniman *et al.* 2008). Ruang-areerate and Kittayapong (2006) were able to introduce a double virus infection of the wAlbA and wAlbB strains into *Ae. aegypti* by microinjecting adults. The *Wolbachia* used to transfect *Ae. aegypti* was sourced from Australian laboratory cultures of *D. melanogaster*, maintained in an *Ae. albopictus* cell line for ~240 passages (about 2.5 years) then transferred to an *Ae. aegypti* cell line and cultured for another 60 passages. Stable infection in live *Ae. aegypti* was achieved by embryonic microinjection (McMeniman *et al.* 2008).

Wolbachia infections in arthropods can be removed by exposure to antibiotics such as tetracycline and rifampicin or by heat treatment (Min and Benzer 1997; Dutton and Sinkins 2005). van Opijnen and Breeuwer (1999) found 71% of the two-spotted spider mite *Tetranychus urticae* were free from infection after rearing at 32°C for four generations, and after six generations infection was completely removed. It indicates that temperature is a key factor in determining the frequency of *Wolbachia* infections in field insect populations. Kyei-Poku *et al.* (2003) found that tetracycline treatment could eliminate *Wolbachia* from the wasp *Urolepis rufipes* (Hymenoptera: Pteromalidae) in four generations while heat treatment at 34°C required six generations. Wiwatanaratnabutr and Kittayapong (2006) reported that *Wolbachia* found in *Ae. albopictus* and reared at 37°C had significantly lower densities compared to the ones reared at 25°C.

CHAPTER 3

ELIMINATE DENGUE PROJECT IN INDONESIA

The Eliminate Dengue Project (EDP) Global is a multi-country research program conducted in Australia, Vietnam, Indonesia, Colombia and Brazil, as well as India, Mexico, New Caledonia, Fiji, Vanuatu and Kiribati which have recently joined the initiative. The project is led by Monash University, Australia and funded through the Grand Challenges in Global Health Initiative, Bill and Melinda Gates Foundation. In Indonesia, the project is known as the EDP-Yogyakarta (EDP-Yogya). EDP-Yogya research is implemented by the Centre for Tropical Medicine, the Faculty of Medicine, Gadjah Mada University (UGM) and funded by Tahija Foundation, a non-profit foundation established in 1990 in Jakarta. The program is developing a new approach to reduce dengue virus transmission using *Wolbachia* bacteria. *Wolbachia* can reduce the capacity of mosquito in transmitting dengue virus to human.

Wolbachia is a natural bacterium present in insects and is passed on through the eggs from one generation to the next generation. The bacterium is present in **60%** of all the different species of **insects** around us. However, it is not found in the *Aedes aegypti* mosquito, the primary vector for dengue diseases. *Wolbachia* was first observed in *Culex pipiens* (Diptera: Culicidae) in 1920 and has been recently known to have a Cytoplasmic Incompatibility (CI) effect. CI results in unsuccessful development of embryo (Stouthamer *et al.* 1999). The results of the risk analysis conducted by the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) in 2011 indicated that *Wolbachia* is safe for humans, animals and the environment.

In 2008, the EDP Global researchers successfully transferred *Wolbachia* from a fruit fly into *Ae. aegypti*. In the laboratory, the bacteria proved to be able to inhibit dengue virus development. The test for assessing *Wolbachia*'s potential in reducing dengue diseases has been conducted through the release of *Wolbachia*-infected *Ae. aegypti* in residential areas in a number of countries including Australia (Yorkeys Knob, Gordonvale, Cairns, Queensland, Machans Beach, Babinda, Parramatta Park, Edge Hill/Whitfield, Westcourt and Townsville), Vietnam (Tri Nguyen island and Nha Trang mainland), Indonesia (Nogotirto, Kronggahan, Jomblangan, and Singosaren), Brazil (Tubiacanga and Jurujuba) and Colombia (Paris).

3.1 EDP-Yogya Research

In July 2011, EDP-Global collaborated with Gadjah Mada University and Tahija Foundation to examine the method for dengue haemorrhagic fever control using *Wolbachia* in Yogyakarta, Indonesia. Yogyakarta Province was selected due to its high incidence of DHF with more than 55 cases per 100 populations. The EDP-Yogya research in Indonesia was designed to be implemented in the following Phases:

3.1.1 Phase I: Safety and Feasibility of *Wolbachia* Release in Yogyakarta (October 2011 – September 2013)

Phase I was aimed at testing the safety of *Wolbachia* technology and EDP Yogyakarta feasibility in conducting the research. Activities and assessments conducted during this phase included:

a. Development of infrastructure and capacity building of staff

The "Tahija Foundation" diagnostic laboratory was located in Gedung Radiopoetro 2nd Floor of the Faculty of Medicine of UGM. The laboratory was qualified with BSL-2 (*Biosafety Level 2*). Staff members had been provided with specific training in dealing with pathogenic infection. All procedures related with infectious agents were conducted in the biological safety cabinet (BSC).

The laboratory was equipped with Light Cycler PCR (LC-PCR) for real-time quantitative PCR (qPCR) that allowed the screening of *Wolbachia* and dengue in a very relatively brief time from a large number of mosquito samples.

The construction of insectarium for insect rearing started in early January 2013 in Sekip complex of UGM. The insectarium was the site for the production or breeding of *Wolbachia*-infected *Ae. aegypti* which were used in the release as well as in entomological-related researches.

The arrangement of the number of staff members involved in the project was conducted following the development of EDP Yogya research stages. Some capacity building activities were provided including General Laboratory Training that covered topics on Environmental Health and Safety and Biosafety and Spills Training, which were organised by World Biohaztec Pte. Ltd, Singapore. Training on qPCR method and serology by the supervisors from EDP-Global and OUCRU Vietnam. A number of staff members in the diagnostic unit, mosquito rearing and entomology unit were also provided with special training in Vietnam and Monash University. Transfer of technology was conducted by EDP Global supervisors in relation to the Diagnostic Laboratory and insectarium. During the first year of the research, two EDP Global supervisors were based in Yogyakarta to develop the standard infrastructure and laboratory system together with EDP Yogya staff members.

b. Screening on the presence of *Wolbachia*

Screening of wild colony of insects was conducted to give the evidence that *Wolbachia* was really present in most insects commonly found around us, both indoor and outdoor, in farming plots and plantations, especially in the research areas of Sleman and Bantul. The result of the screening indicated that *Wolbachia* were present in 21 species (50 %) out of the 42 species insects tested. The insects in which *Wolbachia* is present include *Aphis cerana* (honey bee), *Aedes albopictus* (mosquito) and *Drosophilla melanogaster* (fruit fly). The results of the research was in line with the result of the previous research which proved that *Wolbachia* was commonly found in insects in Indonesia (Narita *et al*, 2007; Lohman *et al*, 2008; Wenseleers *et al*, 1998).

c. Analysis of genetic similarity between *Wolbachia* in mosquito and *Wolbachia* in their original host, *Drosophila melanogaster*

The examination was conducted to demonstrate genetic similarity (close association between strains under examination) of *Wolbachia* using polymorphic markers. The test would determine if the *Wolbachia* strain that was isolated from *D. melanogaster* flies in Indonesia has similarity with the *wMel* strain introduced into *Ae. aegypti*. Sampling of *D. melanogaster* flies was conducted in release areas and around Yogyakarta region using banana, guava, water melon and papaya as fruit traps. Samples were also obtained from *BGtrap*. A comparison was made between the *Wolbachia* found in local *D. melanogaster* and the *wMelPop* and *wMel* strains cultured in local *Ae. aegypti* in EDP-Yogya insectarium. The procedures for identification of the trains followed the protocols published by Riegler *et al*. (2005). The results of the research indicated that insertion fragments and VNTR that were polymorphic between *Wolbachia* strains were identical between *wMel* from the mosquito samples from Australia and the *Wolbachia* strain from Indonesia.

- d. Analysis of genetic similarity between *Wolbachia*-infected *Ae. aegypti* and local *Ae. aegypti* from Yogyakarta

The research was aimed to study the genetic similarity between local *Ae. aegypti* mosquitoes and local *Wolbachia*-infected *Ae. aegypti* that were the result of backcrossing used in this project. Research samples were wMel *Ae. aegypti* and wMelPop *Ae. aegypti* that were the results of backcrossing and local *Ae. aegypti* mosquitoes in research areas. The analysis was done using micro satellite analysis at the Diagnostic Laboratory of Tahija Foundation, Faculty of Medicine, UGM. The result of the analysis showed that there was not any difference between wMel *Ae. aegypti* mosquitoes with local *Ae. aegypti* mosquitoes from Yogyakarta ($p=0.350$). This indicated that the backcrossing between wMel *Ae. aegypti* and local *Ae. aegypti* from Yogyakarta had turned both population genetics to be homogenous that local wMel *Ae. aegypti* from Yogyakarta was no longer identical with the Australian *Ae. aegypti*.

- e. Vector competence study

Vector competence study was conducted to test the ability of *Wolbachia* in suppressing dengue virus in backcrossed *Wolbachia*-infected *Ae. aegypti*. During the first stage, a test of the local *Wolbachia*-infected *Ae. aegypti* population that were reared in the laboratory was made against the wild type mosquitoes. The results of the test indicated that dengue virus could not replicate within mosquitoes that were infected with *Wolbachia*. Similar analysis was also done to *Wolbachia*-infected *Ae. aegypti* population that had been established for two years in Sleman the research coverage areas. The ability of *Wolbachia* to suppress replication of dengue virus in *Ae. aegypti* mosquitoes was still evident despite their long presence in their natural habitat and population. In general, this initial study supported the theory on the role of *Wolbachia* in suppressing the replication of dengue virus in mosquitoes, and therefore similar role to reduce transmission of virus to humans was also expected.

3.1.2 Phase II: Release of *Wolbachia*-infected *Aedes aegypti* in limited scale (October 2013 – December 2015)

The research aimed at (1) identifying the ability of *Wolbachia* to breed and survive in natural population and (2) identifying the ability of *Wolbachia* in suppressing replication of dengue virus in mosquitoes. The phase II research consisted of two activities: first, the release of *Ae. aegypti* in Nogotirto and Kronggahan of Gamping sub-district, Sleman district during January-June 2014, and second laying groups of *Wolbachia*-infected *Ae. aegypti* eggs in small buckets in Jomblangan and Singosaren of Banguntapan sub-district the district of Bantul during November 2014 to May 2015.

Research was conducted at the Diagnostic Laboratory and EDP Insectarium as well as in the field (Sleman and Bantul). Kalitirto and Trihanggo of Sleman district were used as control areas of mosquito population. In Sleman, the distribution of *Wolbachia* through release of adult mosquitoes was conducted in the houses of residents who approved of the release with a distance between point of release of around 25-50 metres. In each point, some 40-80 female and male mosquitoes were released. Released was conducted 20 times. In Bantul, the distribution of *Wolbachia* was done by handing over or laying mosquitoes eggs in the residence of consenting villagers. The eggs were placed in a bucket filled with water and were fed with fish pellets. Each bucket was laid with 60-120 eggs. The distance between the buckets were around 25-50 metres. The eggs, food, and water were replaced every two weeks. Egg replacement was conducted 12 times or until *Wolbachia* reached more than 60% in three observations in a row. Monitoring of *Wolbachia* was conducted using BGtrap and Ovitrap.

The result of entomological monitoring in Sleman district (>3 years after the release) and Bantul (almost 2.5 years after the release) indicated that the released *Wolbachia*-infected *Ae. aegypti* mosquitoes could breed and survive well (>80%) in the natural habitat. There was also an indication

of the ability of *Wolbachia* in suppressing dengue viruses since local transmission was not detected to occur despite the high frequency and population. It indicated that *Wolbachia*-infected mosquitoes had the ability to suppress the development of dengue virus in their natural habitat and that the mosquitoes did not spread or breed in areas outside of the EDP Yogya research coverage areas. Monitoring of areas outside of the research areas also indicated that *Wolbachia* did not spread and breed in areas that were not covered in research.

During the vector competence analysis at the EDP Diagnostic Laboratory, it was indicated that *Wolbachia*-infected *Ae. aegypti* had the ability to block DENV replication in natural habitat.

Intensive awareness raising and campaign to the local community on DHF diseases, *Ae. aegypti* mosquitoes, *Wolbachia* bacteria and information on the progress of the research has led to local community's acceptance of and media positive response to *Wolbachia* technology as an alternative in DHF disease control.

During Phase II, monitoring was done on the horizontal transmission of *Wolbachia* to other insects by detecting the presence of *Wolbachia* wMel in *Culex* sp. Detection of *Wolbachia* in the mosquito was done every three - six months by taking samples that were trapped in BGtraps. The results of the detection indicated that *Wolbachia* wMel was not found in *Culex* sp., and it was an evidence that there was not any horizontal transfer of *Wolbachia* to other species. The results of serological test of *Wolbachia* in human also indicated that vertical transfer did not occur to human. The test was conducted by taking the blood samples of the research team members who regularly feed *Wolbachia*-infected *Ae. aegypti*.

3.1.3 Phase III: Release of *Wolbachia*-infected *Ae. aegypti* in large scale (January 2016 – December 2019)

Phase III aims at assessing the impacts of the release of *Wolbachia*-infected *Ae. aegypti* in large scale on DHF transmission in Yogyakarta City. Two research designs were employed in this phase, namely quasi experimental research with intervention areas (where *Wolbachia*-infected eggs were laid in 7 urban villages) and control areas (3 urban villages) and Randomized Controlled Trial (RCT) through public randomisation to establish 12 intervention clusters for laying *Wolbachia* eggs and 12 control clusters. The clusters did not necessarily represent administrative areas but served as definite physical geographical border to prevent the spread of mosquitoes to other areas. RCT survey was done in 35 urban villages in Yogyakarta City and 2 villages in Bantul District. A number of initial preparatory researches had been conducted, as follows:

- Seroprevalence (proportion of population with antibody against dengue virus) of DHF among children between 1-10 year in Yogyakarta City.
- Study on mobility of children between 1-10 years old in Yogyakarta City
- Monitoring of *Ae. aegypti* population in Yogyakarta using BGTraps
- Mapping of social situation in Yogyakarta City

In general, the research has obtained the approval from the following:

- Ethical clearance from the Medial and Health Ethical Commission, the Faculty of Medicine, Gadjah Mada University for Phase I, II and III.
- Clearance for the importation of research materials (*Wolbachia*-infected *Ae. aegypti* eggs) from the Agricultural Quarantine Agency, the Ministry of Agriculture of the Republic of Indonesia (Phase I)
- Operational permit for the conduct of the research from Yogyakarta Special Province, Yogyakarta City, Sleman District, and Bantul District Local Governments, which is updated every 3 months during the research period.
- Written consent of the local community on the release of *Wolbachia*-infected *Ae. aegypti* in areas around their homes. Consent was provided on individual basis in Sleman district and collective or community consent was provided from local community in Bantul district and Yogyakarta City)
- Written consent of the owners of the houses where BG Traps were installed
- Verbal consent from the owners of the houses in Bantul and Yogyakarta City who were deposited with buckets for laying mosquito eggs
- The plan for contract agreement with the Government Community Clinic (Puskesmas) and the Yogyakarta City Health Office for the recruitment of patients during Phase III (not yet)
- Material Transfer Agreement between Monash University and Gadjah Mada University as the research agency.

There are five main components in the implementation of EDP-Yogya research:

1. Involvement of Local Community and Stakeholders
Local community are given the guarantee that they will be provided with sufficient information on the research through a range of communication, information and educational media. Local community are also encouraged to participate and be actively involved in the research. EDP-Yogya has also established a Stakeholders Inquiry System for stakeholder reporting and responses to accommodate and respond to inputs and feedbacks from local community. On regular basis, stakeholders at the national, provincial, district and village levels are also provided with information concerning the progress of the research.
2. Mosquitoes Rearing
Rearing of *Wolbachia*-infected *Ae. aegypti* is aimed at ensuring the feasibility of the mosquitoes to be released and their ability to breed in their natural habitat.
3. Entomological monitoring
Release and monitoring of *Wolbachia*-infected *Ae. aegypti* population and monitoring of the frequency of *Wolbachia* in research areas and the vicinity. Monitoring is conducted using BGTraps, Ovitrap and GAT Traps for trapping mosquitoes.
4. Diagnostic test
Identification of the presence *Wolbachia* in *Ae. aegypti* from the research areas and the vicinity. A series of tests are later conducted on dengue virus infected mosquitoes.
5. Surveillance
Surveillance of DHF cases in research/release areas.

Phase II of the EDP Project showed a satisfactory result. a. However, further research should be conducted before proceeding to Phase III because analysis of potential risks should be conducted to anticipate potential harm that may occur before the large scale release of *Wolbachia*-infected *Ae. aegypti*. The risk analysis should cover all risks associated with the release of *Wolbachia*-infected *Ae. aegypti*. The analysis should address, for instance, if the release may lead to considerable harm and loss and affect the economy, social welfare, and public health. In addition, consideration should also be given to mosquitoes control measures in the future or adverse changes of biological vectors, *Wolbachia* or even dengue virus itself in the release areas as compared to the current situation.

CHAPTER 4

OBJECTIVES AND METHODS OF RISK ASSESSMENT

4.1 Objectives and Scope

The risk assessment was conducted in Indonesia using a Risk Analysis Framework developed by the Australian Office to the Gene Technology Regulator (OGTR). The risk assessment was aimed at identifying the Indonesia context of the possibilities of adverse impacts on the safety of the community and the environment associated with the release of *Wolbachia*-infected *Ae. aegypti*. The assessment covered all possibilities and scenarios of unprecedented harm that may occur within the next 30 years. To meet this aim, the risk assessment covered three main components, as follows: *Ae. aegypti* as disease vector, dengue virus as disease causing pathogen, and *Wolbachia* as the main factor responsible for reducing the prevalence of Dengue Haemorrhagic Fever (DHF) in Indonesia. **Risk** is defined as an event of particular level of severity and is measured by the multiplication of the potential occurrence of a specific event (**likelihood**) with the level of consequence or impact resulted (**consequence**). In simple equation, **risk = likelihood x consequence**.

Risk assessment was conducted to evaluate the factors that influence the ecology of vectors; social, cultural and economic impact of the release; and mosquito management efficacy; and the public health. The endpoint of the assessment was to address the question whether the release of *Wolbachia*-infected *Ae. aegypti* would “**cause more harm**” or not. Therefore, possibilities were identified concerning the likelihood that the release of *Wolbachia*-infected *Ae. aegypti* will “cause more harm” to the mosquitoes ecology, dengue virus and *Wolbachia*, efficacy of mosquitoes management, standards of public health, and the social, cultural and economic conditions of the local community in release sites as well comparison of the current condition with the next 30 years.

The Risk Assessment were designed to address the following questions:

- Which hazards can happen?
- What could go wrong?
- How could harm/adverse impact occur?
- How likely is the harm to happen?
- How serious could the harm/adverse impact be?
- What is the level of the risk?

4.2 Risk Assessment Framework

In general, a **risk assessment** is the process for estimating the potential impacts of chemical compound, physical, microbiological or psychosocial hazards to human population or a given ecological system within a given condition and a given period of time. There are different models of risk assessment and definitions of terminologies on the assessment of risks associated with the release of *Wolbachia*-infected *Ae. aegypti*. The assessment covers several components, including hazard identification, likelihood of risk, consequence of risk, and level of risk estimation (Figure 4.1).

4.3 Expert Elicitation

Expert Elicitation is the synthesis of the opinions of the experts in different expertise on particular uncertainty due to insufficient data as a result of physical constraint or lack of resources. Expert elicitation is basically an agreement on a particular method. Expert elicitation is often used in

researching into rare events. The processes involved in expert elicitation include the integration of empirical data, scientific prediction based on scientific considerations, and identification of any potential results and possibilities that may emerge. From the processes, experts will commence with identification and assessment of the empirical evidence related with any hazard published in highly reputable journals. What expert elicitation does is generally quantifying uncertainty. In this risk assessment, expert elicitation is used as the main approach in soliciting and synthesizing expert opinions concerning the problems associated with hazards and damage to humans and the environment. In addition, the risk assessment refers to the results of researches conducted by the EDP in Phase I and II.

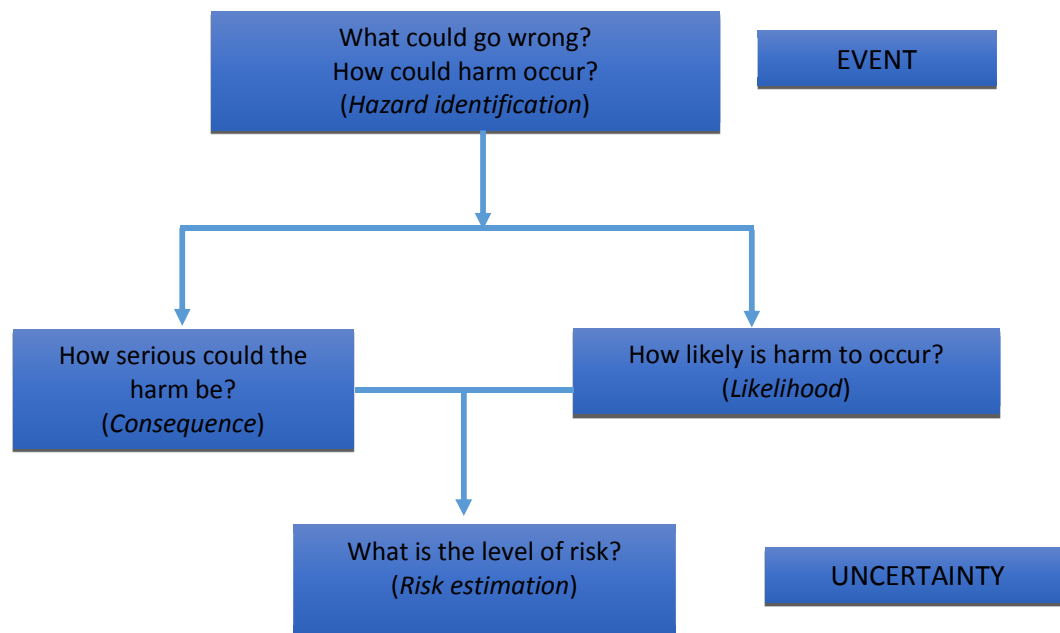


Figure 4.1 Risk assessment framework.

4.4 Bayesian Belief Network (BBN)

The risk assessment was done using the Bayesian Belief Network (BBN) approach. BBN was used for developing a framework for the risk analysis and combine expert judgment with conditional probabilities to determine the risk value for the endpoint. BBN is a probabilistic model described in the form of Directed Acyclic Graph (DAG) to demonstrate the probabilistic link between any given events (Neapolitan 2003). The Bayesian theorem is basically showing that future events can be predicted using any previous events that have happened

The BBN was constructed using GeNIe 2.0, a software package developed and distributed by the Decision Systems Laboratory, University of Pittsburgh (<http://genie.sis.pitt.edu/>). The BBN consisted of two main components, namely DAG and conditional probability table (CPT). DAG consists of nodes and links. **Node** represents the variables being observed that are connected with the **links** to show indications of conditional dependence. A link between node A (**parent node**) and node B (**child node**) shows that A and B are functionally related or that A and B are statistically correlated. Each child node (i.e. a node linked to one or more parents) contains a **conditional probability table** (CPT).

The **CPT** shows the conditional probability for the node being in a specific state given the configuration of the states of its parent nodes. The Bayes theorem is applied according to the values in the CPT. When networks are compiled, changes will happen in the probability distribution for the states at node A which are reflected in changes in the probability distribution for the states at node B.

Bayes' theorem is used to calculate the conditional probability at each node of a hazard under observation. In the software, within each node the outcomes at the previous nodes are given and eventually an absolute probability for the final outcome is derived, which is calculated by taking the product of all conditional probabilities. BBN is used in 2 practical components

- **Network** (graph theory): The connection in a graph depicting the relationship between the variables. The application of this connection helps draw a net of nodes, in which the relation among them is causally established.
- **Probability**: a conditional probability is the probability of one event if another event occurred. This application supports populating the likelihood of each node.

In some risk assessments there will be a range element of judgment with different beliefs based on prior knowledge of each experts. The Bayesian approach incorporate these two factors into the risk assessment by using simulations with different weightings so that prior knowledge, assumptions and judgments can be formulated and explicitly used in the risk assessment. This approach can be as valid as conventional statistic techniques for estimating probabilities.

Boz 1: Bayes Theorem

IF E (event) happens

THEN H (Hypothesis) happens {prob. P}

Example:

*If the road is damaged, traffic jam will **often** happen*

$$p(A/B) = \frac{p(A \cap B)}{p(B)} \quad p(A \cap B) = p(B \cap A)$$

IF the road is damaged

$$\text{T} \quad p(B|A) = \frac{p(B \cap A)}{p(A)} \quad p(A|B) = \frac{p(B|A) \times p(A)}{p(B)}$$

4.5 Steps of the Risk Assessment in Indonesia

The risk assessment of the EDP-Yogya research was the mandate of the national stakeholders meeting held on 12 February 2016 (Figure 4.2). To meet the mandate, the Ministry of Research, Technology and Higher Education (Kemenristekdikti) and the Ministry of Health (Kemenkes) have collaborated to establish an independent core team of experts that were going to responsible for the risk assessment. The process for the establishment of the expert team was facilitated by Kemenristekdikti and also invited the participation of Ditjen P2P, Balitbangkes, AIPI and DRN. The meeting was conducted on 07

April 2016 and 19 April 2016 to identify, select and confirm the candidates of the independent core expert team. From the 14 candidates who were identified, five were selected to become the members of the expert team (Team 5) from a number of universities in Indonesia. The 5-members team or Team 5 consisted of Prof. Dr. Ir. Damayanti Buchori, MSc from Bogor Agricultural Institute as the Chair, and four members, i.e Prof. Dr. drh. Upik Kesumawati Hadi, MS from Bogor Agricultural Institute, Prof. dr. Hari Kusnanto Joseph, SU, DrPH from Gadjah Mada University, Yogyakarta, Prof. Dr. dr. Aryati, MS, SpPK(K) from Airlangga University, Surabaya and Prof. dr. Irawan Yusuf, MSc, PhD from Hasanudin University, Makassar. The 5-members core team have expertises in *Ae. aegypti*, dengue virus, environmental health and risk assessment methodology.

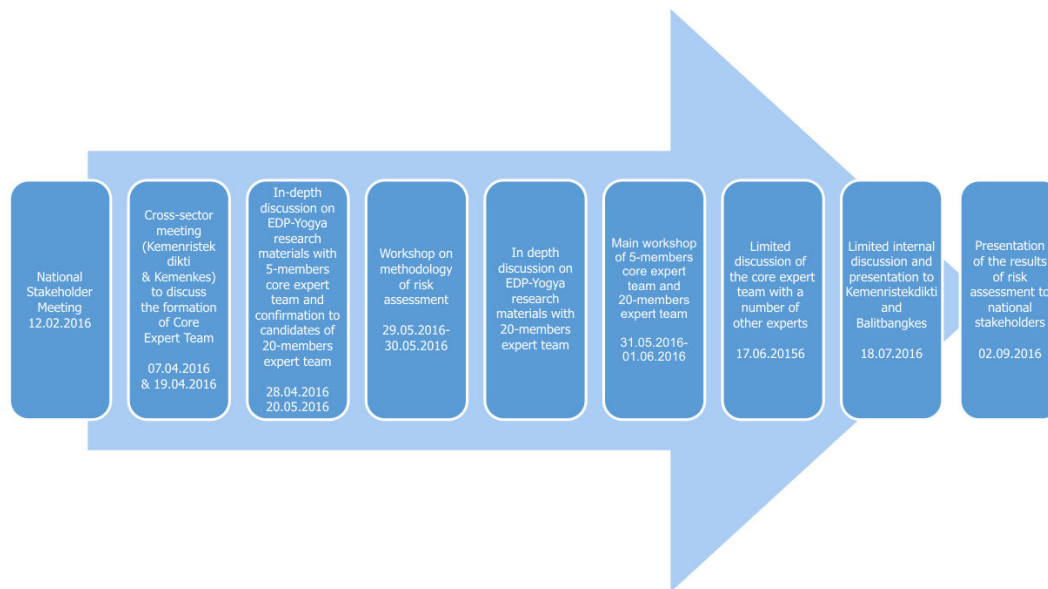


Figure 4.2 Sequence of events of risk assessment on the release of *Wolbachia*-infected *Ae. aegypti*.

Kemenristekdikti later facilitated the Team 5 to have in-depth understanding on EDP-Yogya research through discussions and/or visits to EDP. In addition, the Kemenristekdikti also requested further nomination for the expert team to become a team of 20 experts (Team 20) to Team 5, AIPI and Balitbangkes. The request resulted in identification of 32 names with different range of expertise, who were later invited by the Kemenristekdikti to become members of the expert team. From the nominated 32 names, 22 experts confirmed their availability to become the Team 20. Team 20 consisted of experts in different range of expertise, namely economy and socio cultural, ecology, vector management, and public health.

Further explanation was provided to Team 5 in Yogyakarta, starting from *Wolbachia* safety, release of *Wolbachia*-infected *Ae. aegypti* in Australia up to blood feeding observation, *Wolbachia*-infected *Ae. aegypti* eggs, system for the observation of *Wolbachia*-infected *Ae. aegypti* as well as the locations for the release of *Wolbachia*-infected *Ae. aegypti* during Phase 2. In addition to that, a comprehensive explanation was conducted on 29-30 May 2016 by the Australian team, i.e. Prof. Scott O'Neill from Monash University on the progress and results of EDP-Global research, as well as Dr. Paul de Barro, and Dr. Justine Murray from the Commonwealth Scientific Industrial Research Organisation (CSIRO), who explained about the RA to ensure implementation of the risk assessment together with the Team 20.

The workshop on risk assessment on *Wolbachia*-infected *Ae. aegypti* was attended by the experts in Team 5 and Team 20. During the main workshops, out of the 22 names of experts who confirmed their

availability, 19 experts were present (and from then on the team was referred to as Team 19). The workshop was conducted to elicit opinions from all participants to identify the adverse impacts or hazards associated with the release of *Wolbachia*-infected *Ae. aegypti*. Discussion with Team 19 was led by Team 5 and last for 2 days during 31 Mei-1 June 2016. The Team 19 was divided into 4 smaller groups on Ecology, Socio-cultural and Economy, Mosquito Management Efficacy, and Public Health. Each small group was led by 1-2 members of the Team 5. During the meeting, the 4 small groups also made identification of hazards. Each hazard from each group was identified by assessing their risk/consequence if the hazard happened by assigning a scoring value of 0-1. The scoring became the basis for defining the hazards. During the following day, discussion continued with determining the likelihood ratio of each hazard. Likelihood ratio was defined as the extent to which a hazard was likely to happen using the scoring value of 0-1. The resource persons from Australia, Dr. Paul de Barro and Justine Murray, provided the information and clarification required by the core expert team and the expert team.

The next meeting was conducted by inviting 1-2 experts to represent each group (Ecology, Socio-Cultural and Economy, Mosquito Efficacy Management, and Public Health) together with Team 5, who were later changed into Team 10 in Jakarta on 17 June 2016. In the meeting, further discussion was held on the results of the workshop in Yogyakarta related to the scoring assigned for the consequence ratio and likelihood, and a discussion on the Bayesian network. The discussion also involved Justine Murray through teleconference to clarify questions, difficulty, or differences in understanding the scoring.

The next meeting of the Team 5 was done on 18 July 2016 in Jakarta to finish the assessment as well as present the results of the evaluation and methods of the risk assessment to the Director General of Kemenristekdikti and Head of Balitbangkes. During that meeting, a conclusion from the risk assessment was drawn that there would be a negligible risk with the release of *Wolbachia*-infected *Ae. aegypti* to ecology, economy and social and culture, vector efficacy management, and public health. The results of the risk assessment were later presented in the national wider stakeholders forum, which was facilitated by the Kemenristekdikti on 02 September 2016.

Stages of risk assessment is presented in box 2:

Box 2: Stages in risk assessment

Stage 1: Preparation

- **Step 1:** Endpoint (issue) identification
- **Step 2:** Selection of experts
 - Scope and structure of elicitation
 - Design of elicitation protocols
 - Selection of experts
 - Preparation for elicitation session

Stage 2: Expert elicitation

- **Step 3:** identification and mapping of hazards
- **Step 4:** risk likelihood
- **Step 5:** risk consequence
- **Step 6:** estimation risk level

Stage 3: Reporting and follow-up

- **Step 7:** writing, consultation with experts and disseminating report

4.6 Limitations and Uncertainties

There were possibilities of limitations and uncertainties concerning the results of the risk assessment due to the attributes of the methodologies used in the assessment. It is clear that there are differences on how experts perceive risks. Several factors can influence this different interpretation, including: personal experience of the adverse impact under observation, social cultural background and beliefs, ability to exercise control over a particular risk, access to information from different sources, a tendency to over-estimate very low risk and sometimes to under-estimate very high ones. At this stage of the process, a risk must be considered a potential risk because it is not known if it occurs in actual ecosystems. In addition, there are also probabilities of different perception of risks due to limited knowledge on *Wolbachia* and infected mosquitoes. Ecosystem is a complex matter relating to biodiversity and natural environment, containing different kinds of living organisms, a large number of species, about which little is known. Other difficulties in risk assessment include unavailability of *Netica software* that in each and every meeting, calculation in the risk analysis was conducted manually.

Chapter 5

PROBLEM FORMULATION, HAZARD IDENTIFICATION AND MAPPING

5.1 Introduction

Problem formulation was carried out to identify the end point adverse events associated with the release of *Wolbachia*-infected *Aedes aegypti*. This was followed by hazard identification and mapping. Participants of the elicitation workshop agreed on the endpoint that the release of *Wolbachia*-infected *Ae. aegypti* would “cause more harm” than the existing dengue-vector control efforts without biologically altering the mosquitoes. The time-frame estimated for the risk that “cause more harm” associated with the release of *Wolbachia*-infected *Ae. aegypti* to occur would be 30 years.

5.2 Methods

Participants of the elicitation workshop were grouped according to the four identified components of “cause more harm”, namely negative effect to ecology, reduced efficacy of mosquito management, worse standard of public health, and negative social-cultural and economic impact. Each group discussed all possible hazards leading to each of the components of “cause more harm” in the context of releasing *Wolbachia*-infected *Ae. aegypti* for a duration of 30 years.

The expert elicitation on hazard identification and mapping was undertaken in several steps: i) identification of events, ii) determination of possible states of the events, iii) development of the list of hazards and agreed definitions, iv) consensus about all hazards and their definitions. A **hazard** is a potential source of harm for persons, communities and ecosystems. Hazard and risk are often used interchangeably; however, hazard can be defined as “an act or phenomenon that has the potential to cause harm to humans or what they value” (Severtson and Burt 2012). All the hazards were mapped into a tree, representing causal relationships.

5.3 Results

The technical experts and other participants of the workshop identified 57 hazards (nodes), which were mapped into sub-trees of the four components of “cause more harm” and altogether were combined leading to the “cause more harm” endpoint. Overall there were 57 hazards, including the endpoint, that were identified.

5.3.1 Ecology

Wolbachia strains in *Ae. aegypti* are expected to suppress viral transmission, invade mosquito populations, and persist without loss of viral suppression ability and not interfering with other control strategies. To achieve these objectives, evolutionary constraints are needed and viral suppression may be hindered by the inability of *Wolbachia* to spread due to altered host fitness effects (Hoffmann *et al.* 2015). The success or failure of *Wolbachia* in suppressing disease depends on its interactions with the ecosystem.

Participants in the elicitation workshop decided to include 18 ecological hazards and **ecological effect** as the **end point**. All of the hazards were defined (Table 5.1) and mapped into a sub-tree diagram that is related to the negative effects of ecological change (Figure 5.1).

Table 5.1 Definition of hazards associated with the negative effect of ecological change when *Wolbachia*-infected *Ae. aegypti* mosquitoes are released

No	Hazard	Definition
1	Change in biodiversity	Change in composition, structure and biodiversity of mosquitoes, virus and <i>Wolbachia</i> in their natural habitat
2	Transfer of <i>Wolbachia</i> genome to invertebrates	Horizontal transfer of <i>Wolbachia</i> or some of their genomes to other invertebrates
3	Transfer of <i>Wolbachia</i> genome to vertebrates	Horizontal transfer of <i>Wolbachia</i> or some of their genomes to vertebrates
4	New mosquito species evolve	New species or strain of mosquito evolves
5	Selection for more virulent arboviruses	Selection of more virulent arboviruses causing higher morbidity/damage and mortality
6	Change in genetic variation	Change in genetic diversity of <i>Ae. aegypti</i> species in nature
7	Vector change	Change in vector species, including vector density, behaviour, biology and reproduction
8	Increased vector density	Increased average number of mosquitoes per household due to possible changes in fecundity, longevity and vector population dynamic
9	Increased host biting	Increased frequency of host biting by <i>Wolbachia</i> -infected <i>Ae. aegypti</i>
10	Female biased Sex Ratio	Change in sex ratio, skewed to female mosquitoes, which leads to an increase in the mosquito vector population
11	Increased Mosquito Host Range	Increased number of hosts other than humans enhancing the likelihood of acquiring new viruses or pathogens
12	Increased Filarial Fitness	<i>Wolbachia</i> -infected <i>Ae. aegypti</i> can enhance the filarial fitness to the mosquito
13	Replacement of dengue vectors	<i>Ae. aegypti</i> would no longer be dengue vector, replaced by other mosquito species or other organisms
14	Transfer of other pathogens	<i>Ae. aegypti</i> may be able to transfer other arboviruses or parasites e.g. Zika or filariasis
15	Ecosystem service change	Changes in ecosystem structure, functions or services
16	Environmental change	Change in geographical distribution, niche of <i>Ae. aegypti</i> habitat and ecosystem services in certain areas
17	Ecological niche	Changes of ecological niche of <i>Ae. aegypti</i> from being a domestic species to a broader or alternative niche.
18	Geographic distribution	Changes in geographical distribution of <i>Ae. aegypti</i>
19	Ecological effect	Ecological impact of <i>Wolbachia</i> -infected <i>Ae. aegypti</i> release

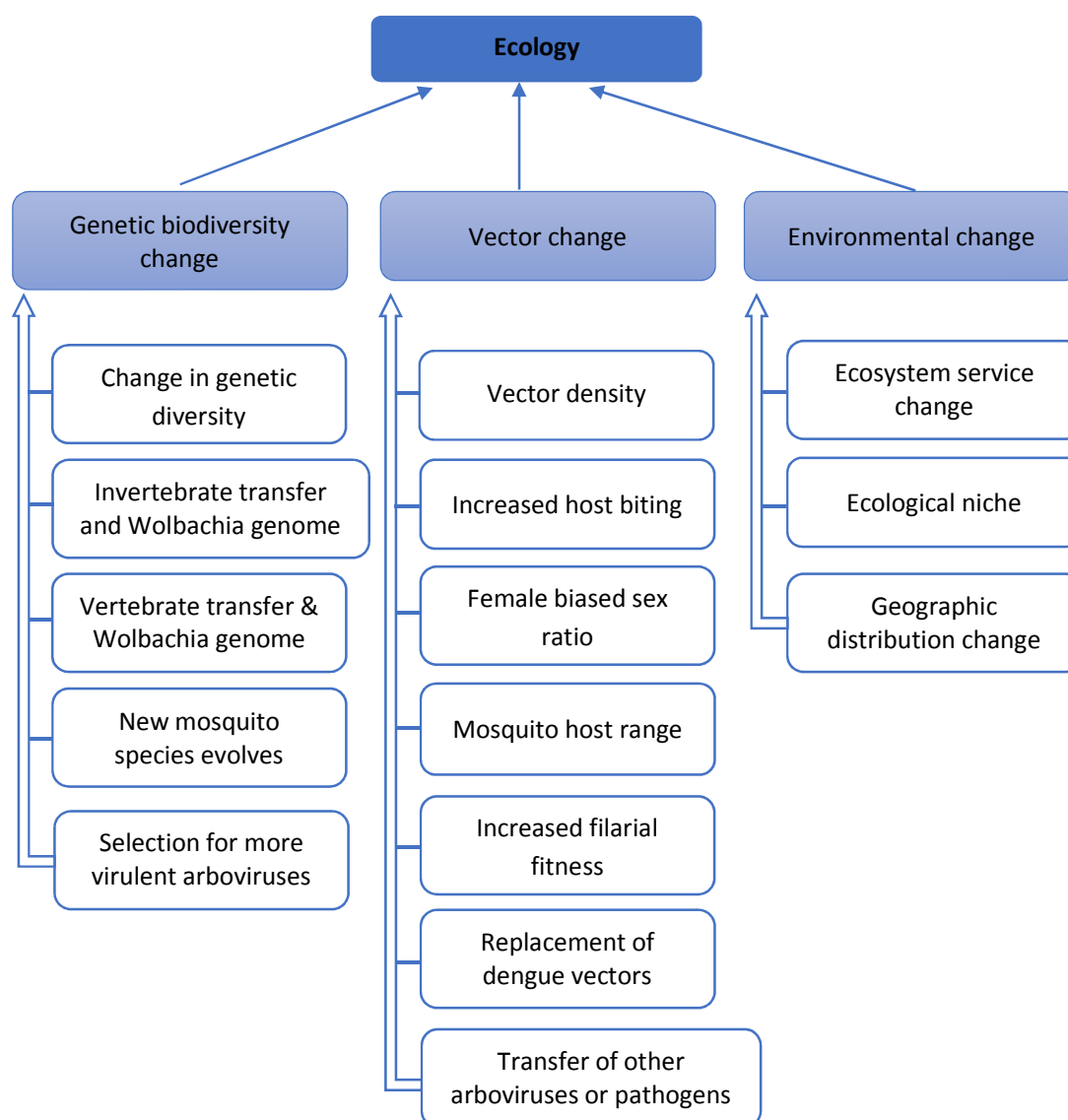


Figure 5.1 Mapping of hazards leading to negative ecological impact as the end point

5.3.2 Mosquito Management Efficacy

Sustainable vector control interventions are necessary to significantly reduce dengue transmission (WHO 2012). Community participation in dengue control needs to be continuously promoted to ensure that community can successfully maintain their own household environment free from dengue vector (Tapya-Conyer *et al.* 2012). The release of *Wolbachia*-infected *Ae. aegypti* may discourage preventive measures by the community through mosquito management. In addition, it can also increase difficulty in *Ae. aegypti* control due to the development of cryptic breeding sites (Dieng *et al.* 2012). Increased complacency at the household level as a result of *Ae. aegypti* control may result in the increase of mosquito density, frequency of mosquito biting and greater possibility of dengue transmission. Community's complacency as a result of the release of *Wolbachia*-infected *Ae. aegypti* can lead to decreased caution on the presence of *Ae. aegypti*. Experts participating in the workshop identified the **endpoint (efficacy of mosquito management)** and 11 hazards (Table 5.2), and a map of the subtree diagram (Figure 5.2), associated with reduced efficacy of mosquito management.

Table 5.2 Definition of hazards associated with decreased efficacy of mosquito management

No	Hazard	Definition
1	Household control	Changes in dengue vector control activities by household members
2	Increased complacency	Decreased community participation in dengue vector control due to perceived comfort and safety
3	Avoidance strategy	Changes in normal mosquito avoidance strategies
4	Transmission of other pathogens	Increased transmission of pathogens other than dengue virus
5	Difficulty for mosquito control	Increased difficulty in mosquito control due to changes in breeding places of <i>Wolbachia</i> -infected <i>Ae. aegypti</i>
6	Behavior change of <i>Wolbachia</i> -infected <i>Ae. Aegypti</i>	Changes in behaviour of <i>Wolbachia</i> -infected <i>Ae. aegypti</i> related to dengue transmission and breeding places
7	Increased resistance to insecticide	Increased resistance to dose and types of insecticide after <i>Wolbachia</i> -infected <i>Ae. aegypti</i> mosquitoes have been release and established
8	Mosquito strain selection	Emergence of <i>Ae. aegypti</i> with higher vector capacity
9	More dengue infection	Increased transmission of dengue virus
10	Increased biting rate	Increased frequency of host biting by <i>Wolbachia</i> -infected <i>Ae. aegypti</i>
11	Increased dengue virulence	Worse clinical outcomes caused by dengue infection
12	Decreased efficacy of mosquito control	Lack of management efficacy of <i>Ae. aegypti</i> control

Aedes aegypti mosquitoes have limited dispersal capability; however, they are capable of exploiting peridomestic environment and laying eggs, which may be the reason for their ability to withstand prolonged desiccation for up to one year. Mosquito population can be suppressed by reducing breeding sites consistently. Unless consistent source reduction is implemented, *Ae. aegypti* remains to be one of the most invasive mosquito species.

Some issues of concern became the main focus of discussion among the expert team associated with efficacy of mosquito control, as follows:

- There is a concern that release of mosquitoes will render people and the government complacent with the new approach and abandon the established mosquitoes control programs. Mosquito control programs are not only targeted at reducing dengue incidence but also at reducing cases of malaria and chikungunya.

- The expert team also discussed the importance of understanding resistance to insecticides. Application of pesticides is one of the reliable techniques in controlling *Ae. aegypti* mosquitoes. The presence of *Wolbachia*-infected *Ae. aegypti* has caused some concerns that *Wolbachia* will increase resistance to pesticides. A number of researches indicated that there was an increase in resistance to pesticides in *Wolbachia*-infected *Culex pipiens* (Berticat *et al.* 2002, Duron *et al.* 2006). However, this did not happen in *Wolbachia*-infected *Ae. aegypti*. Endersby and Hoffmann (2012) concluded that *Wolbachia* did not influence the level of resistance to pesticides among mosquitoes. In other words, the success of chemical control is not influenced by the presence of *Wolbachia* in mosquitoes. During the discussion of this particular group, the expert team agreed that the hazard should be included in the group on efficacy of mosquito management.
- The prepresence of *Wolbachia*-infected *Ae. aegypti* has also led to concerns on changes of community's behaviour. Changes of behaviour are suspected to contribute to difficulties in mosquito management processes.
- Increased biting rates that can increase dengue infection was also an important factor discussed by the expert team for Efficacy of Mosquito Management. The team identified that such possibility should be taken into careful consideration.

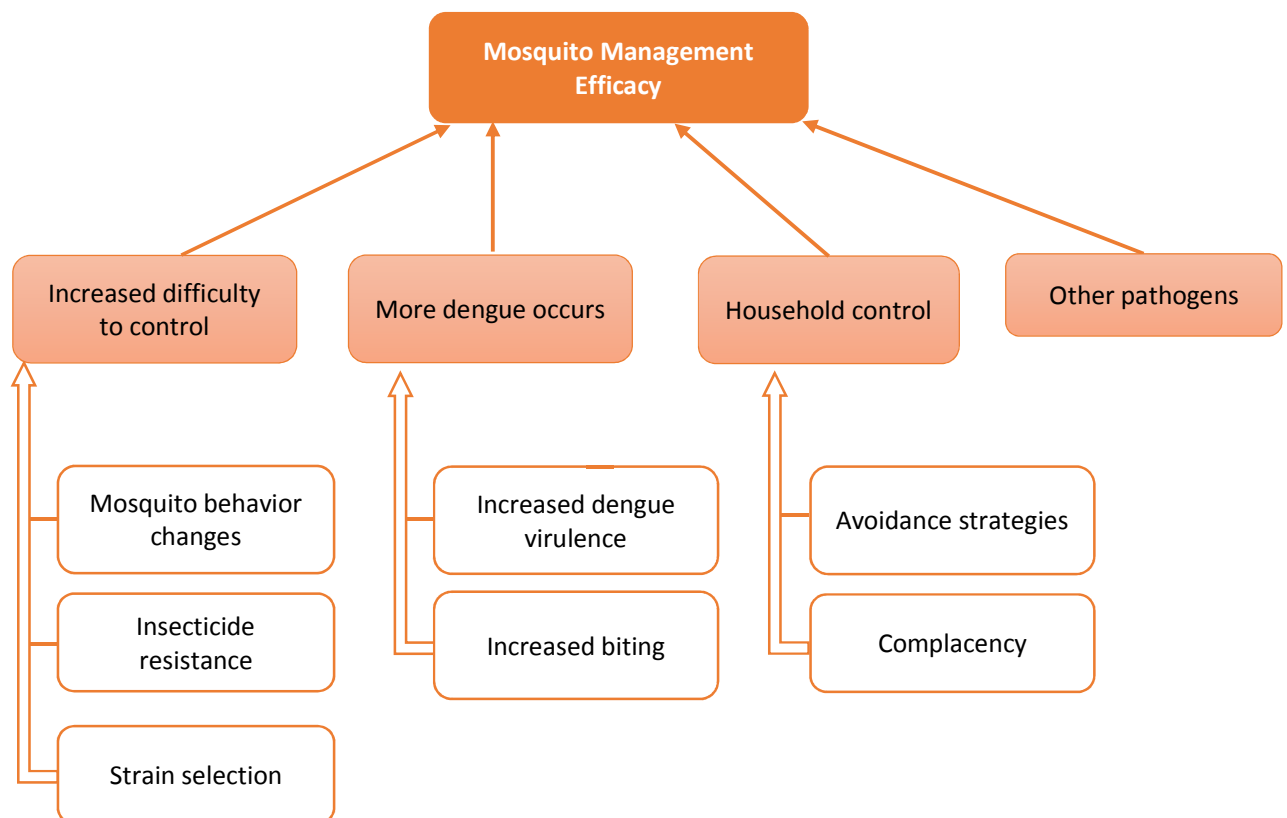


Figure 5.2 Mapping of hazards with decreased mosquito management efficacy as the endpoint

5.3.3 Public Health

Experts discussed what would be the impact of the release of *Wolbachia*-infected *Ae. aegypti* on the health of the population in the release sites. The endpoint was worse health status of the community living within or in the vicinity of the release sites. There are 13 hazards, namely increased dengue viral transmission, more cases of dengue diseases, more severe dengue diseases, higher risks to infections by other pathogens, and interference with other dengue control measures, which may lead to lower standard of public health (Table 5.3). The hazards range from insect biting nuisance (Cooke *et al.* 2002) to higher incidence of more serious dengue diseases. The sub-tree diagram describing lower standard of public health indicate the complexities of dengue transmission (Figure 5.3).

Table 5.3 Definition of hazards associated with lower standard of public health when *Wolbachia* infected *Ae. aegypti* mosquitoes are released

No	Hazard	Definition
1	Increased dengue transmission	The rate of dengue transmission increases compared to the situation before the release of <i>Wolbachia</i> -infected <i>Ae. Aegypti</i>
2	Dengue evolution	Dengue virus evolves so that its transmission would be more effective
3	Increased dengue vector competence	<i>Ae. aegypti</i> becomes a more capable vector in transmitting dengue virus
4	Increased feeding frequency	<i>Ae. aegypti</i> takes blood meal more frequently
5	Increased mosquito density	Average number of <i>Ae. aegypti</i> per household would be higher
6	Increased biting	Increased frequency of host biting
7	Nuisance biting	Increased pest status of <i>Ae. aegypti</i> , due to increased tendency to associate with people, uninhabited houses, severity of bites and mosquito population density
8	Non-dengue vector competence	Increased vector competence as disease agents of other diseases than dengue
9	Host preference	Increased variety of host animal infested with <i>Ae. aegypti</i>
10	More dengue cases	Increased number of dengue cases
11	Transmission of non-dengue pathogens	Increased capability of <i>Ae. aegypti</i> to transmit pathogens other than dengue virus
12	Increased severity of disease	More severe manifestations of dengue infection, and more older people affected by the disease
13	Interference with other dengue control	The presence of <i>Wolbachia</i> -infected <i>Ae. aegypti</i> has caused disruption to the larva free index indicators as part of the dengue control program
14	Lower standard of public health	The overall standard of public health would be worse

The expert team for Public Health identified the same hazards with the ones in the Efficacy of Mosquito Management group in terms of the evolution of *Wolbachia* and mosquitoes, rate of biting, and mosquito resistency. This is an indication of common perception of those different factors in the Risk Assessment.

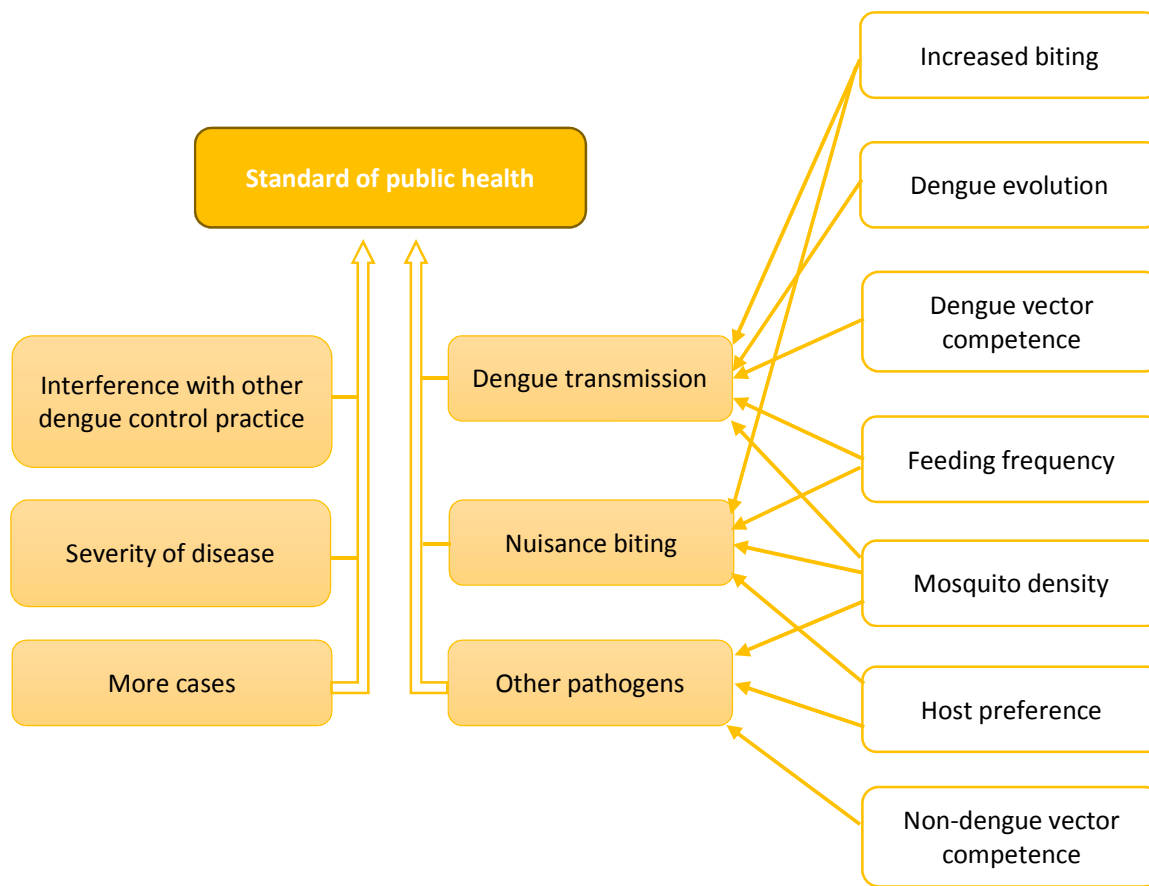


Figure 5.3 Mapping of hazards with lower public health standard as the endpoint

5.3.4 Socio-Cultural and Economic Impacts

According to the experts participating in the elicitation workshop, adverse economic impact of the release of *Wolbachia*-infected *Ae. aegypti* is associated with loss of income, more expenses, increased health care cost and decreased tourism rate due to the release of *Wolbachia*-infected *Ae. aegypti*. Socio-behavioral changes may also occur due to adverse social media reports related to the release such as social fear, social conflict, and movements to the site of the release because of perceived safety from dengue infection (causing increased human population density), scapegoating and class action (Table 5.4). Initially the group of experts focused their discussion on social and economic aspects only, but as discussion went on, they also took into consideration the cultural impact associated with the release of *Wolbachia*-infected *Ae. aegypti*.

Perceived effectiveness of vector control is important, as community-based integrated approach is the most successful intervention to control dengue by taking into consideration the local socio-cultural wisdom and ecological as well as epidemiological factors (Erlanger *et al.* 2008). In real life, the socio-behavioral and economic factors are intertwined. There were 12 hazards and endpoint (economic and socio cultural) mapped as separate sub-trees (Figure 5.4).

Table 5.4 Definition of hazards associated with negative socio-cultural and economic impacts associated with the release of *Wolbachia*-infected *Ae. aegypti* mosquitoes

No	Hazard	Definition
1	Health care cost	The cost for health care in general will increase
2	Tourism	Local and international tourism will be affected by the release
3	Loss of income	Individual and corporate businesses will lose lost their incomes
4	Increased expenses	Increased expenses due to monitoring and controlling mosquitoes
5	Economic change	Decreased income and increased expenses will negatively change the economy
6	Scapegoating	Negative collective defense mechanism as technology fails
7	Migration	Changes in destination of migration area due to perceived safety or perceived threat
8	Adverse media	Negative social media messages leading to concerns among the public
9	Social conflict	Contradictory opinions in the society based on different knowledge and beliefs
10	Class action	Legal actions from individuals, groups, communities, and community organizations
11	Social fear	Collective mental confusions due to unintended consequences without proper assurance
12	Socio-cultural change	Negative social behaviour and deterioration of local wisdom, such as increased social isolation and decreased community participation
13	Economic and socio-cultural change	Adverse economic and socio-cultural change due to the release of <i>Wolbachia</i> -infected <i>Ae. aegypti</i>

The expert team incorporated conflict and class action factors into the subtrees given that in the beginning of the EDP project journey, a social conflict occurred with the local community in Karangtengah of Nogotirto village, Gamping sub-district, Sleman district, which ended up in the class action lawsuit (Sugarman 2014). Current situation indicated that the social conflict had been addressed properly that no more conflict occurred with the local community. However, for caution, the expert team integrated social conflict as an important faktor for consideration in the Risk Assessment. The expert team also integrated adverse socio-cultural changes resulting from the release of *Wolbachia*-infected *Ae. aegypti* as one component of hazards. Socio cultural changes may occur when, for instance, the release of *Wolbachia*-infected *Ae. aegypti* undermine the collective behaviour among local community in addressing problems. Economic loss was also integrated as one adverse impact if the release of *Wolbachia*-infected *Ae. aegypti* lead to a decline in the number of travellers visiting the area out of fear.

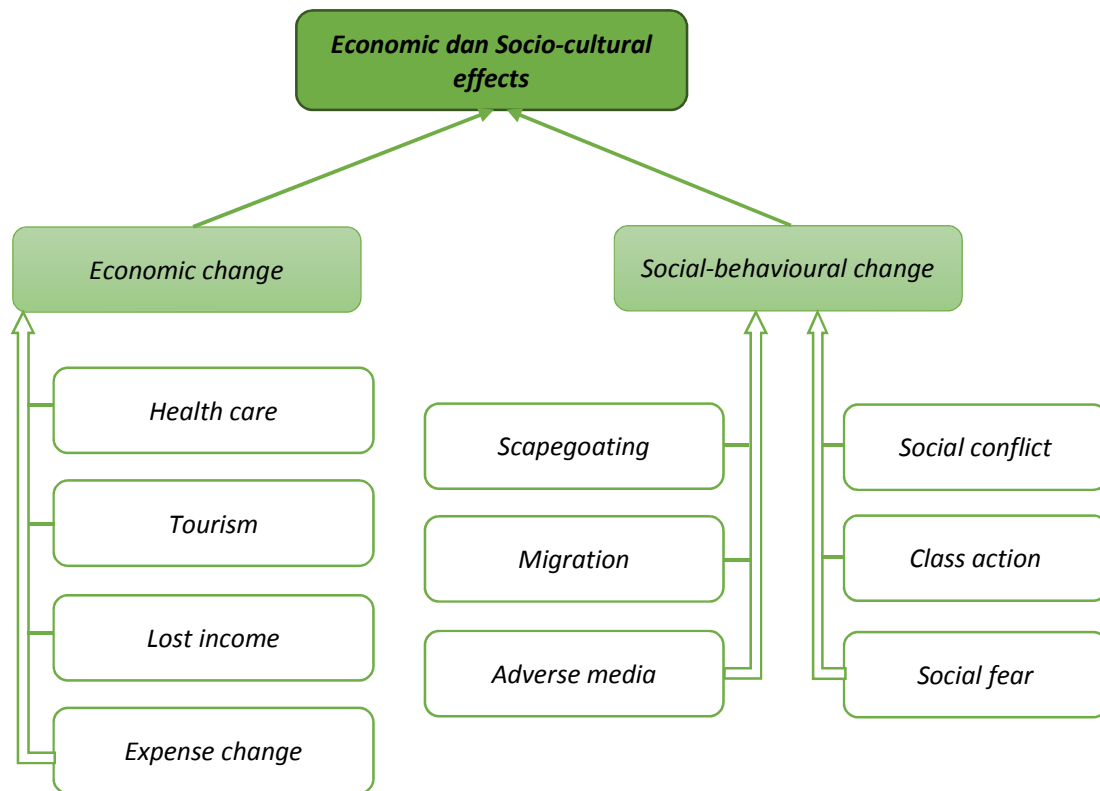


Figure 5.4 Mapping of hazards that lead to negative economic and socio-cultural impacts as endpoint.

CHAPTER 6

EXPERT ELICITATION ON BAYESIAN BELIEF NETWORK LIKELIHOODS

6.1 Introduction

Following the hazard identification and mapping, the likelihood and the consequence of the hazards to occur were estimated. The consequence of a particular hazard indicates the potential severity of the hazard while likelihood indicates the level of probability that a hazard can actually occur. Other experts and participants involved in the elicitation processes assigned the likelihood of the occurrence of each hazard. The aim of the likelihood elicitation was to estimate the likelihood that the release of *Wolbachia*-infected *Aedes aegypti* would “cause more harm” compared to the existing activities in dengue vector control using the existing regulations and technologies where *Wolbachia*-infected *Ae. aegypti* were not released. The previous chapter (Chapter 5) describes the four groups of 57 hazards identified by the experts, namely: adverse impact to mosquito ecology, worse economic and socio-cultural impacts, lower standard of public health, and decreased efficacy of mosquito management. A number from zero (0) to one (1) was assigned to estimate the likelihood that each hazard may occur. The smaller the value of the likelihood, the less likelihood that the hazard will occur; a zero value means that the hazard will never occur while the value 1 indicates that a hazard will certainly occur.

6.2 Methods

Bayesian Belief Network (BBN) was used to obtain probabilistic relationship between events and to provide graphical representation of those events (as nodes) with a number of possible states, and a directed acyclic graph from the parent node (cause) to the child node (effect). The absence of an arc between two nodes means that no conditional probability table (CPT) can be defined. The results of a BBN were often convincing and conclusive, even when sufficient data was not available (Langseth and Portinale 2007). BBN has often been used in representation of knowledge and support in decision making under uncertainty (Mkrtchyan *et al.* 2015). BBN is very suitable to be used in estimation of probabilities of occurrence of hazards caused by the release of *Wolbachia*-infected *Ae. aegypti* as a result of uncertainty (due to lack of knowledge on the long term benefit of the presence of *Wolbachia* in natural environment).

6.3 Results

Discussions about the likelihoods of BBNs in this risk assessment took place among 4 groups of experts or informants. The groups discussed issues pertaining to the ecology, socio-cultural and economic impacts, standard of public health, and efficacy of mosquito management.

6.3.1 Ecology

In the discussion in the Ecology component, all possibilities concerning changes to vector, virus and bacteria that may cause more harm in the next 30 years were discussed. There were at least 18 hazards identified that may lead to adverse impacts to the ecology as a result of the release of *Wolbachia*-infected *Ae. aegypti*. Key factors that may contribute to changes in ecology were incorporated, including genetic changes to virus mosquito and bacteria that may lead to the evolution of new species. Changes to mosquito density, behaviour, and biology and changes in preferences of hosts besides human that may lead to transmission of new pathogens by *Ae. aegypti*

were also included. In addition, the expert team also calculated changes to *Ae. aegypti* environment and ecological niche (Table 6.1 and Figure 6.1).

Table 6.1 Estimated probability of the ecological hazards

No	Hazard	Estimated probability of the hazard (0% – 100%)
1	Change in genetic biodiversity	1.00
2	Transfer of <i>Wolbachia</i> genome to invertebrates	0.10
3	Transfer of <i>Wolbachia</i> genome to vertebrates	0.10
4	New mosquito species evolve	0.10
5	Selection for more virulent arboviruses	1.00
6	Change in genetic variation	1.00
7	Vector change	10.2
8	Increased vector density	5.00
9	Increased host biting	1.00
10	Female biased Sex Ratio	1.00
11	Increased Mosquito Host Range	0.10
12	Increased Filarial Fitness	0.10
13	Replacement of dengue vectors	5.00
14	Transfer of other pathogens	0.10
15	Ecosystem service change	0.10
16	Environmental change	1.14
17	Ecological niche	2.00
18	Geographic distribution change	4.00
19	Adverse ecological effect	4.74

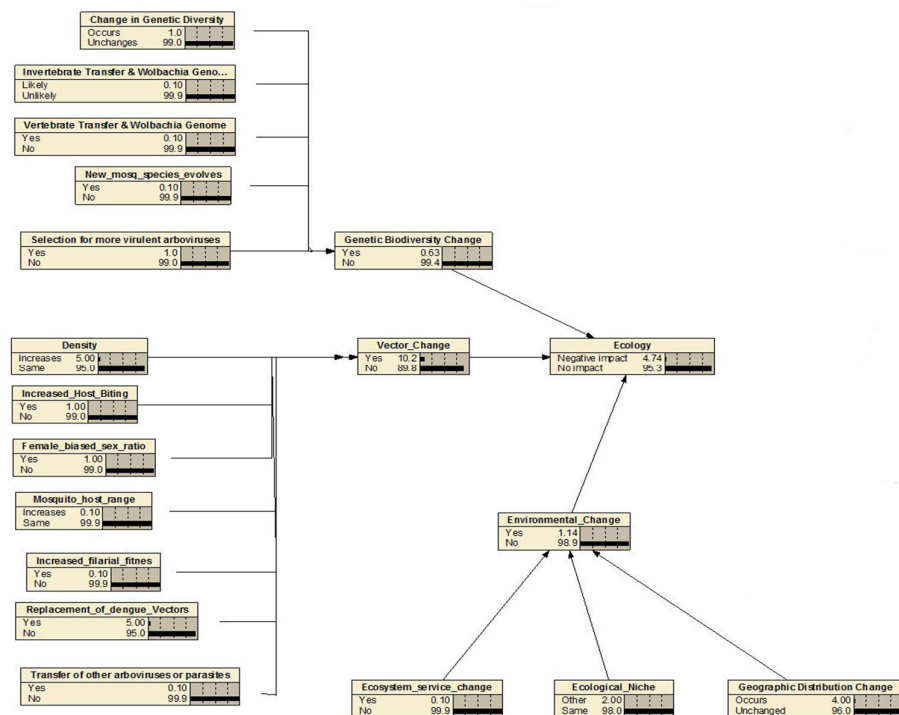


Figure 6.1 Sub-tree diagram describing ecological aspect of Bayesian probability for “causing more harm”

In this component, at least there were 7 hazards identified with a very low score of likelihood of 0.10%, including transfer of other pathogens. The expert team assigned a very low score of likelihood since *Ae. aegypti* that is not infected by *Wolbachia* are anthropophilic, endophagic and endophilic in nature, which means that insect that attack human tend to stay indoor and rest indoor after taking blood meal (the time when blood meal digestion take place and mosquito eggs develop) (WHO 2009b, Scott 2010). Such patterns of behaviour have made mosquitoes the right agents for viral pathogenic transmission by arthropods or parasites that are responsible for diseases among humans and animals. *Ae. aegypti* is the primary vector of several arboviruses that attack humans such as yellow fever virus, DENV (Flaviridae) (Kyle and Harris 2008), chikungunya virus (Togaviridae) (Morrison 2014) and Zika virus (Flaviridae) (Dutra *et al.* 2016, ecdc 2016, Muktar *et al.* 2016). Researches showed that in addition to suppressing DENV transmission and reduced life span or fecundity of *Ae. aegypti* mosquitoes, *Wolbachia* (wMel strain) is also known to reduce the mosquito's ability to transmit Zika virus (Aliota *et al.* 2016, Dutra *et al.* 2016).

Among the hazards in the ecology component, the highest score of likelihood to happen was assigned to vector change (10.2%). Vector change is defined as the changes in the density, behaviour, biology and reproduction of vectors. Researches indicated that the presence of *Wolbachia* in *Ae. aegypti* mosquitoes suppresses mosquito's growth rate due to cytoplasmic incompatibility (CI) and causes changes in behaviour that can suppress dengue viral transmission. Reduced mosquito growth rate in *Ae. aegypti* that is infected by wMelPop strain has been indicated by reduced fecundity and egg viability in *Ae. aegypti* (McMeniman *et al.* 2009, McMeniman and O'Neill 2010). In addition, wMelPop also causes changes of behaviour in *Ae. aegypti*, as indicated by Turley *et al.* (2009) and Moreira *et al.* (2009b) which showed that wMelPop-infected mosquitoes fed on less blood meal than mosquitoes that are not infected. Despite that, experts assigned a relatively high score of likelihood because the finding by Weeks *et al.* (2007) indicated that after 20 years, naturally occurring *Wolbachia*-infected *Drosophila simulans* exhibited 10% increase in fecundity over flies that were not infected by *Wolbachia*. In other words, the bacterium characteristic has changed from being parasitic to more mutualistic.

The release of *Wolbachia*-infected mosquitoes is one of the mosquito-borne vector control widely known as population-replacement technology (Scott and Morrison 2003). The conceptual basis for the population-replacement technology is the reduced transmission of the diseases through the introduction of a gene into a mosquito population that confers resistance to the pathogen (James 2000). Therefore, vector density is one of the factors that are closely related to the evaluation and application of the control technique. The experts decided that there was 5% likelihood of an increase in vector density. This is a relatively high score since on the contrary of the results of a study that indicated a decline in mosquito population density which invaded by *Wolbachia*-infected mosquitoes (Hoffmann *et al.* 2011). The study also showed that *Wolbachia*-infected *Ae. aegypti* can survive in naturally-occurring *Ae. aegypti* population after it was release in a number of areas in Australia. However, experts assigned a score of 5% for the likelihood of an increase in vector density as a caution to the release of *Wolbachia*-infected mosquitoes. Likewise, the elicitation experts decided to assign 5% of likelihood for replacement of dengue vector to describe changes in the next 30 years. In addition, the influence of selection pressure and mortality rate to *Wolbachia*-infected *Ae. aegypti* fitness should be ensured. Overall, the likelihood of an adverse impact to ecology was estimated to be very low (4.74%).

6.3.2 Mosquito Management Efficacy

The expert team identified 11 hazards (Chapter 5.3.2) and assigned scores ranging from 1 to 15.9% (Table 6.2 and Figure 6.2) for the likelihood of the hazards to occur. One of the hazards that we should be aware is the increased complacency among community members as a result of *Ae. aegypti* mosquito control at the household level. Increased complacency as a result of *Wolbachia*-infected *Ae. aegypti* control technique is a threat to the success in vector mosquito control strategies at the household level (Murray *et al.* 2016). Until now most vector mosquito control measures are community based, where the successes are influenced by the members of the community. In Guantanamo, Cuba, a community-based environmental control of *Ae. aegypti* embedded in a routine, government-led vector control program, significantly reduced the infestation level of *Ae. aegypti* (Vanlerberghe *et al.* 2009). In Indonesia, successful community-based vector control such as the 3M (burying, draining and covering) program will increase community's complacency towards the control program, which will eventually lead to a decline in vector control measures that are conducted by the community themselves. Such decline can lead to a failure in suppressing vector population and the rate of dengue viral transmission. It is evident that even in the absence of *Wolbachia*, a decline in dengue cases will increase community complacency and consequently reduce their awareness and eventually lead to a decline in vector control efforts.

Table 6.2 Estimated probability of reduced efficacy of mosquito management

No	Hazard	Estimated probability of the hazard (0% – 100%)
1	Household control	15.9
2	Increased complacency	10
3	Avoidance strategy	5
4	Transmission of other pathogens	9.43
5	Difficulty for mosquito control	2.57
6	Behaviour change of <i>Wolbachia</i> -infected <i>Aedes aegypti</i>	10
7	Increased resistance to insecticide	5
8	Mosquito strain selection	5
9	More dengue infection	8.33
10	Increased biting rate	1
11	Increased dengue virulence	4
12	Decreased efficacy of mosquito control	10.5

The expert team assigned a high score for the likelihood of increased complacency hazard to occur. In addition, issues about accuracy of information from different media sources of information and incorrect interpretation of *Wolbachia*-infected mosquito control strategies may lead to increased complacency. It should be noted that for the next release of *Wolbachia*-infected mosquitoes, relevant parties are expected to provide detailed explanations about how the mosquito control techniques work to the public. Moreover, emphasis should also be given that such measure should go hand in hand with other control techniques to suppress dengue viral transmission.

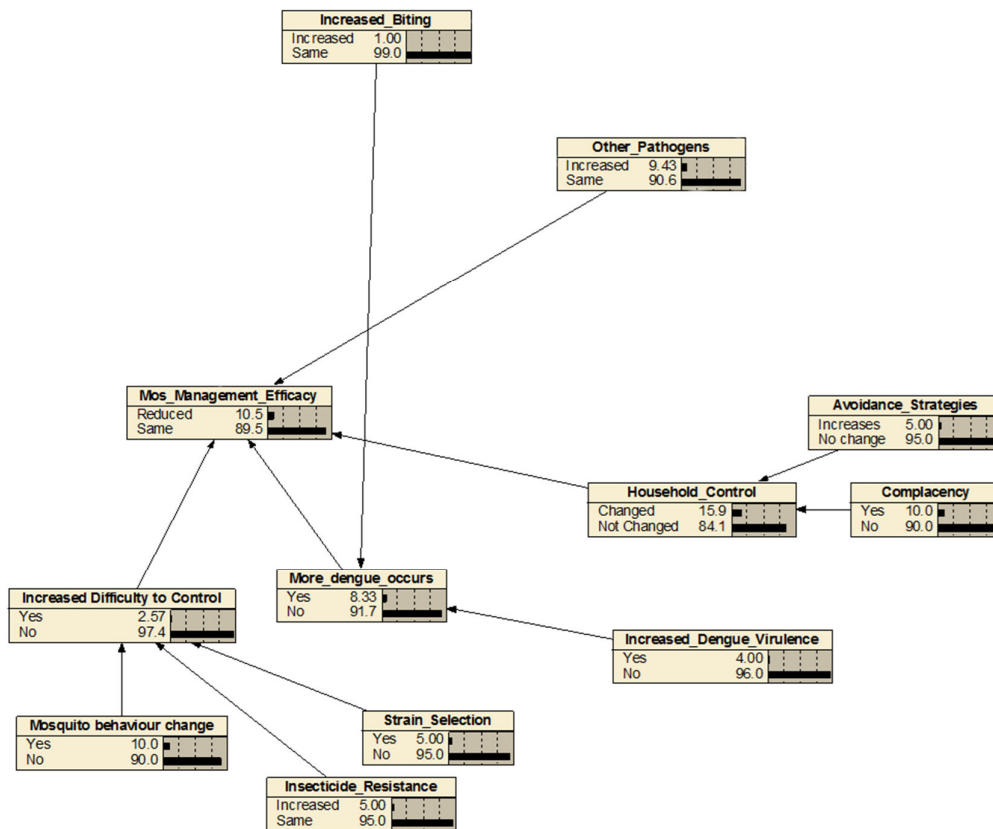


Figure 6.2 Sub-tree diagram describing efficacy of mosquito management as part of the Bayesian probability for “causing more harm”.

The expert team concluded that there was 15.9% probability for increased household control as a result of the release of *Wolbachia*-infected mosquitoes. It means that control measures do increase as *Wolbachia*-infected mosquitoes are released due to possibilities of changes in *Wolbachia*-infected *Ae. aegypti*, which may eventually lead to changes in mosquito behaviour, more virulent selection of dengue viral strain, and *Ae. aegypti*'s resistance to many more types of insecticides.

In principle and from results of recent studies, the release of *Wolbachia*-infected *Ae. aegypti* will not lead to increased household control as the presence of *Wolbachia* in local mosquito population can lead to a decline in vector mosquito population. The expert team decided to include this hazard for further risk assessment and as a precaution in assessing the risk of the hazard for the next 30 years, a high score of likelihood was assigned.

Mosquito resistance to pesticides has been reported by a number of studies in Indonesian, especially resistance to pyrethroid synthetic insecticide (Ghiffari *et al.* 2013) and organophosphates such as temephos and malathion (Prasetyowati *et al.* 2016). Chemical control using insecticides is only one of the techniques at the household level commonly used by the public. Most of these insecticides, widely known as mosquito repellent, contain active substance of D-allethrin, D-transallethrin, deltamethrin, transfluthrin and metoflethrin, which are all pyrethroid insecticides. YLKI (2011) reported the use of the following forms of mosquito repellent pesticides by households: mosquito coils for everyday use (54%), spray/liquid (19%), lotion (17%), electrical tablet (15%) and electrical liquid.

It is widely known that long term and intensive use of one particular insecticide for vector mosquito control can lead to resistance. It is not surprising that many mosquito populations have begun to develop resistance to insecticides. One of the impacts of resistance to insecticides is increased cases of dengue fever in one particular area. However, it takes a long time for mosquitoes to develop such resistance. Soko *et al.* (2015) reported that *Anopheles* mosquito (a vector of malaria) developed resistance to a number of groups of insecticides after 30-40 years use of the insecticides.

The use of wMel and wMelPop-infected *Ae. aegypti* exactly reduces the use of insecticides that it reduces the rate of resistance. Endersby and Hoffmann (2013) reported that the level of susceptibility of *Ae. aegypti* mosquitoes that are infected by wMel and wMelPop strains of *Wolbachia* to insecticides commonly used in mosquito control measures is similar to mosquitoes that are not infected by *Wolbachia*. However, it is worth noted that different result was evident from Echaubard *et al.* (2010), who reported that increase density of *Wolbachia* in naturally *Wolbachia*-infected *Culex pipiens* could increase mosquito resistance to insecticides. It should be noted that *Ae. aegypti* and *Cx. Pipiens* are two different species that what happened to *Cx. Pipiens* may not necessarily happen to *Ae. aegypti* as they have different evolutionary pathways. Based on these facts, the expert team decided to assign a score of likelihood of 5%.

Increased DENV virulence was one of the hazards identified by the expert team with a score of estimated probability of 4%. The level of severity of dengue fever depends on a number of factors, including host, agent, vector and the environment. Bull and Turelli (2013) indicated that many has yet to be revealed about the interaction between the factors that it is difficult to develop a definitive estimation concerning changes of each factor. The researchers further indicated that DENV evolution, *Wolbachia* and mosquitoes could happen as a result of human intervention in suppressing DENV transmission. In theory, virulence of parasitic organism can evolve in response to interventions, including vaccine (Gandon and Day 2003, Mackinnon *et al.* 2008). *Wolbachia* can alter both the viral life history in the mosquito and the mosquito life history. Changes in both factors can theoretically would affect evolution of viral virulence in humans and mosquitoes (Bull and Turelli 2013). Recent epidemiologic and phylogenetic studies indicated that more virulent dengue genotypes have begun to displace dengue viruses with lower epidemiological impacts (Ricco-Hesse 2003).

Transmission of other pathogens was estimated to have a probability of 9.43% to take place since *Wolbachia pipientis* is known as intracellular bacteria found in many species of arthropods and nematodes (Hilgenboecker 2008, Kambris *et al.* 2009). Taylor *et al.* (2005) explained that genetic analysis divides *Wolbachia* into 6 supergroups A-F. Supergroups A and B are *Wolbachia* found in arthropods while supergroups C and D are *Wolbachia* found in filarial nematodes. There is still ongoing debate on whether the six supergroups should be grouped into different species or not while acknowledging the inevitably different characteristics between the six (Taylor *et al.* 2005, Pfarr *et al.* 2007). Besides, *Wolbachia* in arthropods have different role than *Wolbachia* in filarial nematodes (Chapter 2.2.1).

6.3.3 Public Health

The release of *Wolbachia*-infected *Ae. aegypti* may affect the standard of public health in the release areas. There are 13 hazards identified as “lower standard of public health” with 6.96% probability. The probability of the hazards ranges from 1% to 15.1% (Table 6.3 and Figure 6.3). The expert team assigned the highest score of probability of 15.1% to increased dengue transmission.

Table 6.3 Estimated probability of lower standard of public health

No	Hazard	Estimated probability of the hazard (0% – 100%)
1	Increased dengue transmission	15.1
2	Dengue evolution	5
3	Increased dengue vector competence	5
4	Increased feeding frequency	1
5	Increased mosquito density	10
6	Increased biting	1
7	Increased nuisance biting	14.6
8	Non-dengue vector competence	5
9	Host preference	10
10	More dengue cases	1
11	Transmission of non-dengue pathogens	9.43
12	Increased severity of disease	1
13	Interference with other dengue control	1
14	Lower standard of public health	6.96

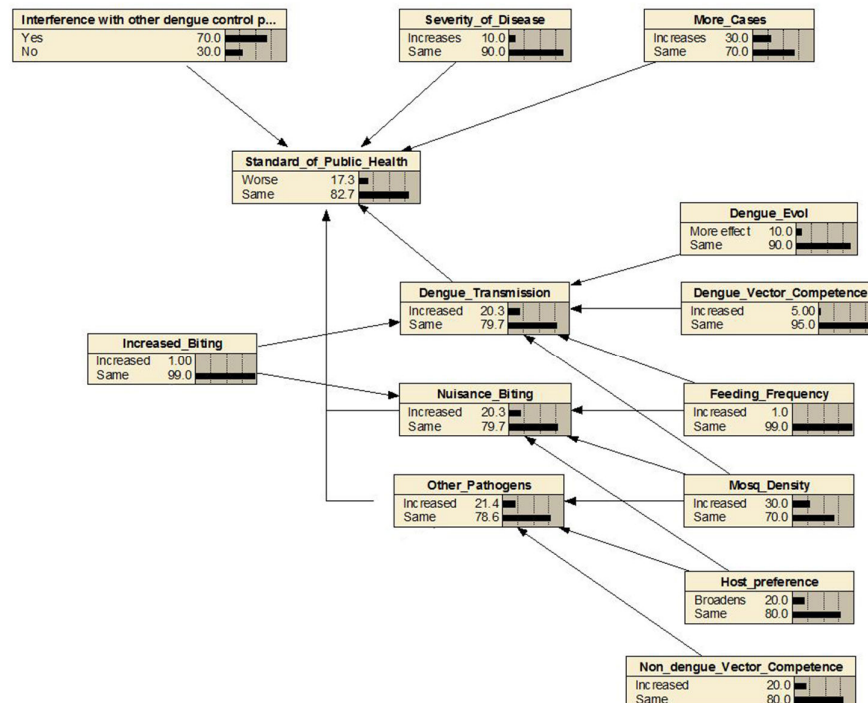


Figure 6.3 Sub-tree diagram describing lower standard of public health as part of the Bayesian probability for “causing more harm”.

Hazard is defined as increased rate of dengue transmission after the release of *Wolbachia*-infected mosquitoes. So far, results of studies on the rate of dengue transmission by *Wolbachia*-infected *Ae. aegypti* have indicated a decline. One of the primary factors that influence the ability of mosquitoes in transmitting DENV is the extrinsic incubation period (EIP). EIP is the developmental time required for virus to reach the saliva glands of the mosquito after an infectious blood meal.

The earlier the virus appears in the saliva, the more opportunities for the mosquito will have to transmit DENV to humans. Ye *et al.* (2015) reported that wMel lengthens the EIP, reduces the frequency of the virus to be transmitted through the saliva. Moreover, the study also showed that *Wolbachia*-infected *Ae. aegypti* mosquito's saliva had less DENV copy compared to wild-type mosquitoes that were not infected by *Wolbachia*. Mosquito salivary gland is the main way for virus transmission. *Wolbachia* is mostly found in mosquito midgut and salivary glands, both very essential in transmitting virus (Zouache *et al.* 2009). Therefore, lower density of DENV in mosquito salivary glands may suggest reduced virus transmission.

In addition to increased dengue transmission, experts also assigned a relatively high score for probability of three other hazards, namely increased nuisance biting (14.6%), increased mosquito density (10%), and transmission of non-dengue pathogens (9.43%). Increased nuisance biting is the hazard associated with mosquito status as pest due to its increasing preference to associate with humans and empty house and severity of bites and density of population, eventually leading to adverse impact to humans. Experts assigned a high score of likelihood since female *Ae. aegypti* is known to have a specific preference restricted to humans (Mousson *et al.* 2012) although so far no report concerning increased biting severity has been reported.

The expert team for Public Health component shared the same concerns with the Ecology component expert team. Both teams identified increased mosquito or vector density as a hazard that we should be aware of. *Wolbachia* can cause various effects on vector fitness, which can lead to changes in size and age distribution of *Ae. albopictus* mosquito larva population in breeding sites (Mains *et al.* 2013). Yeap *et al.* (2011) reported reduced viability of wMelPop-infected *Ae. aegypti* eggs happened when the eggs are held in dried state. This can lead to a drastic decline in mosquito population density in the area until there is reinvasion of other mosquitoes from other areas (Rašić *et al.* 2014).

Other hazards such as increased feeding frequency, increased biting, more dengue cases, increased severity of diseases, and interference with other dengue control were considered by the expert team to have very low probability to happen that they were assigned with a probability of 1% only. A study by Ye *et al.* (2015) confirmed this concerning increased feeding frequency. Ye also reported that although infection of certain strains of *Wolbachia* in mosquitoes did not cause virulent effects, *Wolbachia* infection in mosquitoes may affect saliva production and mosquito behaviour such as feeding frequency.

6.3.4 Socio-Cultural and Economic Impacts

The expert team suggested that the release of *Wolbachia*-infected *Ae. aegypti* might have significant impact on socio-cultural and economic conditions of the community with a probability of 19.2% (Table 6.4 and Figure 6.4). The expert team identified 12 hazards that may happen as a result of the release of *Wolbachia* with probabilities ranging from 2% to 50%. According to Gómez-Dantés and Willoquet (2009), dengue clinical picture, in the context of social and cultural environment, should be considered in addressing vector control strategies and vector surveillance capabilities. During the workshop, social conflict, class action and social fear were estimated to have 50% likelihood of occurrence. The three hazards did actually occur during the release of *Wolbachia* in a number of areas in Yogyakarta (EDP Yogya) (See Chapter 5.3.4). Therefore, the likelihood of the hazard to take place in the next 30 years is very high if the public is not provided with comprehensive and clear information on this vector control technology. Furthermore, adverse media coverage by printing media and social media can become a hazard that lead to another hazard. Therefore, the expert team assigned this particular hazard a score of 40% of likelihood.

Table 6.4 Estimated probability of socio-cultural and economic adverse impacts

No	Hazard	Estimated probability of the hazards (0% - 100%)
1	Increased health care cost	5
2	Decreased tourism	2
3	Loss of income	2
4	Increased expenses	5
5	Economic change	10
6	Scapegoating	30
7	Migration	10
8	Adverse media	40
9	Social conflict	50
10	Class action	50
11	Social fear	50
12	Socio-cultural change	18
13	Economic and socio-cultural change	19.2

The expert team identified the likelihood of increased health cost for dengue when *Wolbachia*-infected mosquitoes are released. This hazard was taken into account as it was considered to be the impact of “more dengue cases” hazard (see Chapter 6.3.3). More severe dengue cases increase social and economic burden to the society. In Puerto Rico, the cost of medical treatment attributed to dengue infection was five times the cost of surveillance and vector control (Halasa *et al.* 2012). Ineffective vector control is a waste of resources since the cost of dengue control activities could be 72% above the cost for the treatment alone (Packierisamy *et al.* 2015). In addition to that, ineffective control can increase dengue transmission. Therefore, an integrated technology is essential to be able to suppress the cost for dengue treatment, including the technique of using *Wolbachia*. Until now, there has not been any evaluation on the social and economic impacts of the release of *Wolbachia*-infected *Ae. aegypti* that caution should be taken in case the technique using *Wolbachia* does not work effectively and to avoid over expectation of the decline of dengue cases.

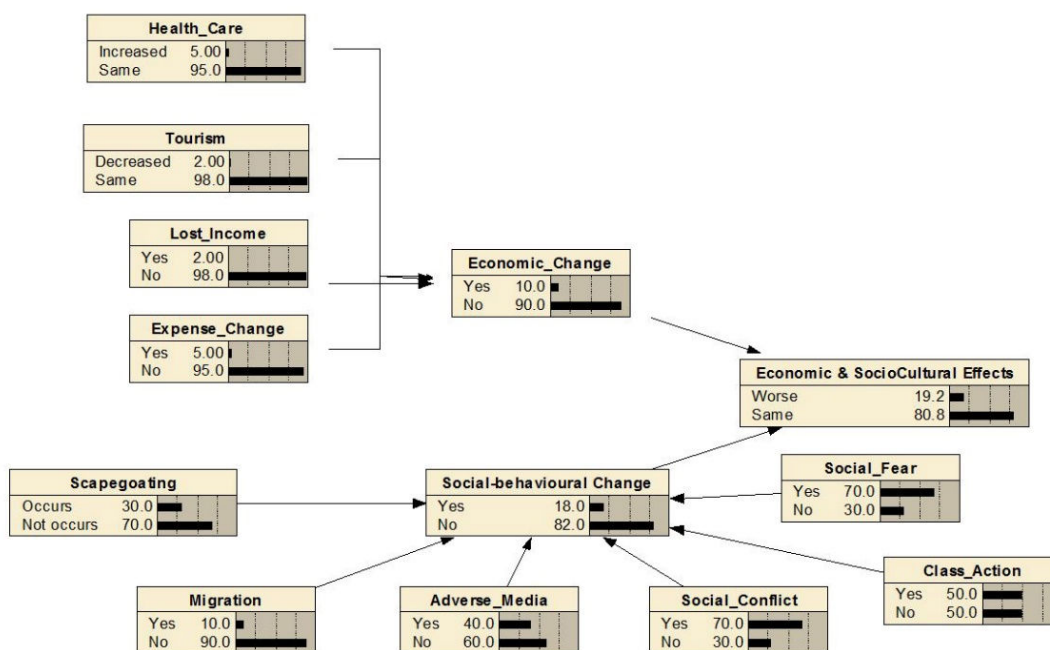


Figure 6.4 Sub-tree diagram describing socio-cultural and economic impacts as part of the Bayesian probability for “causing more harm”.

6.4. Summary

During the Risk Assessment discussion among the expert team members, number of concerns arose that needed attention, such as the social aspect of the release. Experience from the first release by EDP Yogya indicated that pros and cons emerged in release areas that could potentially lead to disharmony among the community. During the expert team discussion, feedback was provided that awareness raising activities were important to disharmony and conflict among the community. Other factors that needed attention were lack of knowledge about the biology and evolution of *Wolbachia*, interaction of *Wolbachia* with other species, and the non-target impact of the release of *Wolbachia*-infected *Ae. aegypti* to the health of the community and the environment. Non-target impact included the probability in increase of filariasis as a result of the release of *Wolbachia*-infected *Ae. aegypti*. One of the relevant questions about this was “would the release of *Wolbachia*-infected *Ae. aegypti* increase the number of filarial cases in the field?”. Based on the results of the discussions and existing scientific evidence, there was not any relationship between the release of *Wolbachia*-infected *Ae. aegypti* and filariasis since *Wolbachia* found in arthropods were distinct from *Wolbachia* found in filarial nematodes.

The results of expert elicitation from a number of workshops identified a total of 57 hazards as a result of the release of *Wolbachia*-infected *Ae. aegypti* with “cause more harm” endpoint. Estimation of cause more harm using BBN indicated that failure likelihood from “cause more harm” is 1.11% (negligible likelihood) (Figure 6.5). The hazards were grouped into the following 4 submodels: “ecological effects” (4.74%) and “standard of public health” (6.96%) with negligible likelihood; submodel “mosquito management efficacy” (10.5%) and “economic and sociocultural effect” (18.3%) with very low likelihood to happen if *Wolbachia*-infected mosquitoes are released to suppress the transmission of DENV.

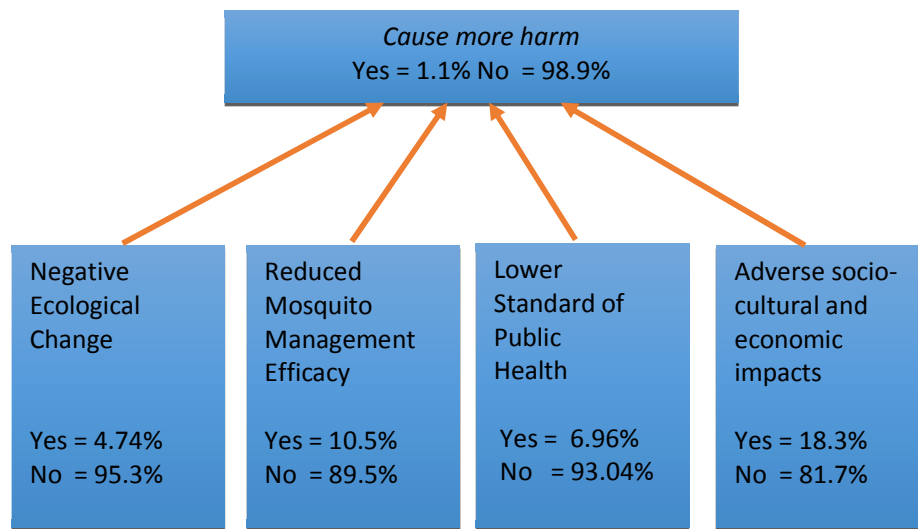


Figure 6.5 Estimated likelihood of the adverse impacts of the release of *Wolbachia* associated with four identified hazards.

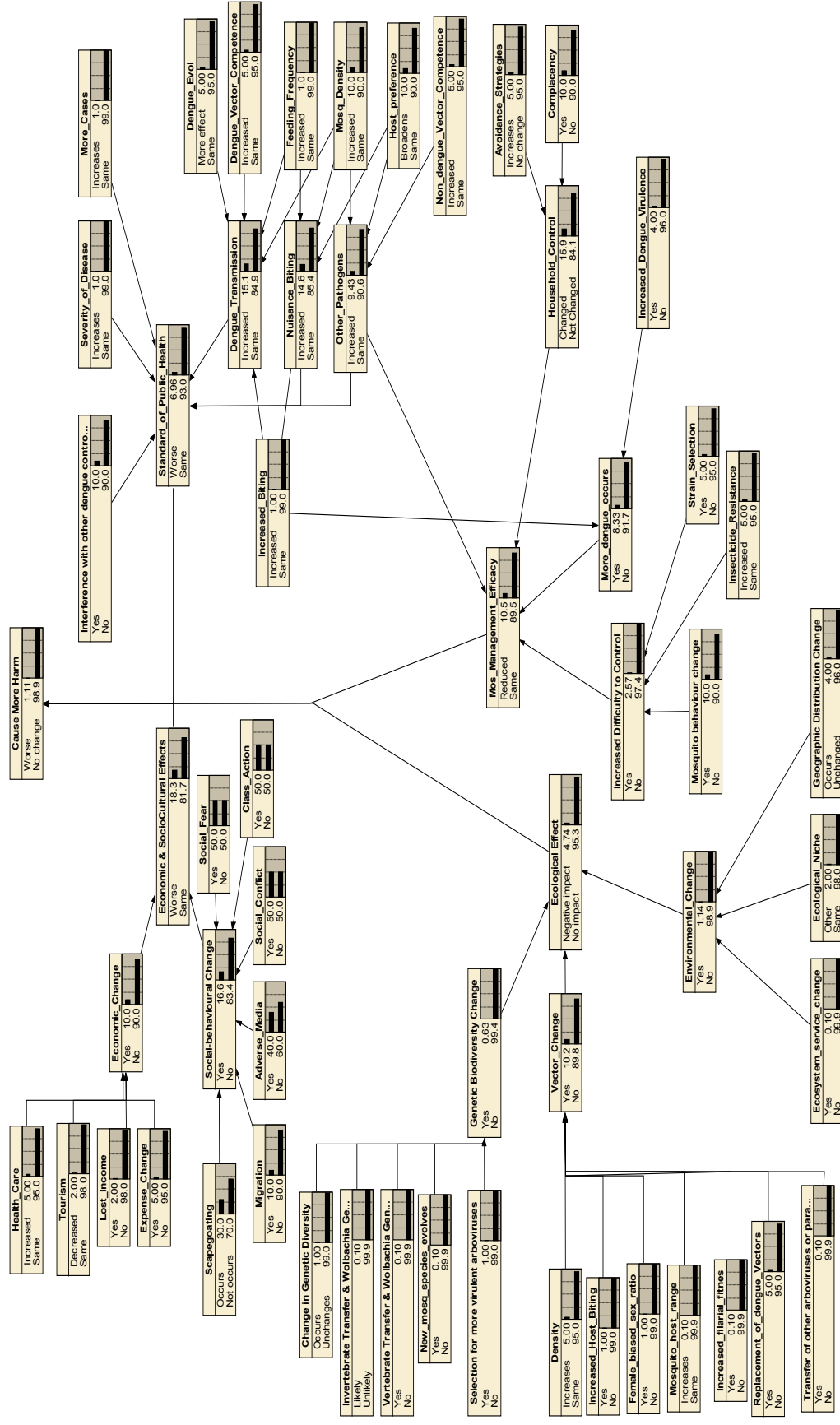


Figure 6.6 Subtree diagram describing the aspects of Bayesian probabilities concerning the ecology, efficacy in mosquito management, standards of public health, and economic and socio-cultural conditions as part of the Bayesian probability for “causing more harm”

CHAPTER 7

EXPERT SOLICITATION ON CONSEQUENCE AND RISK ESTIMATES

The last stage of the elicitation process was the solicitation of the experts on the consequences of the identified hazards. As described earlier in Chapter 3, *Consequence* is defined as the level of severity of any hazard that may likely to happen. The level of severity of the harm is given a scale from 0-1, with 1 being the most severe. Estimations of the consequence should a hazard occur were done through expert solicitation that was held in Jakarta. Ten experts were involved in the process. Overall Risk was calculated after the consequence estimate was reached. The consequence of a hazard was reached through discussions among the experts and consensus building based on expert judgement. The previous Chapter 5 explained about the definition of each hazard that is estimated to be likely to happen as a result of the release of *Wolbachia*-infected mosquitoes while Chapter 6 explained about the likelihood that the hazards can occur based on the existing information. Chapter 7 will discuss expert solicitation about the consequence of each hazard and risk assessment. Risk was determined by members of the core team (Team 5) and the results of the expert solicitation were calculated using the ***Risk = Consequence x Likelihood*** equation (Figure 7.1)

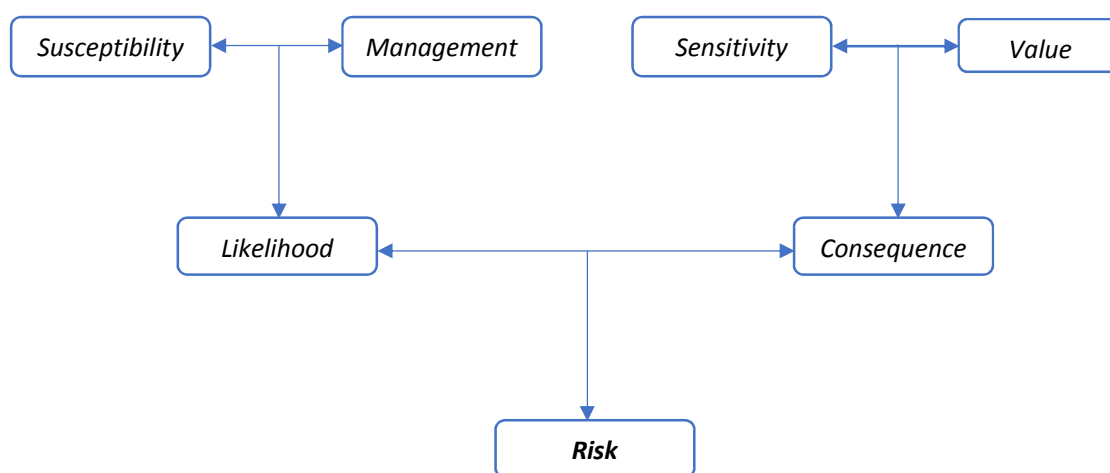


Figure 7.1 Components and flow of risk assessments used by the experts for any identified hazards.

7.2. Methods

The *Australian Risk Assessment Report* and *Vietnam Risk Assessment Report* were used as the reference for determining the scale of the risks and what the scale represented, both qualitatively and quantitatively. The experts then agreed on a scale to score the likelihood and consequence of the identified hazards (Table 7.1).

Table 7.1 Scale for likelihood and consequence estimation

Scale	Negligible	Very Low	Low	Moderate	High	Very High
Probability	0 – 0.01	0.02 – 0.10	0.11 – 0.40	0.41 – 0.74	0.75 – 0.89	0.90 – 1

Definitions of the scale in estimation of *likelihood and consequence* were also discussed and agreed among the experts during the meeting in Yogyakarta. The definitions were based on possible impacts to human health and ecosystem (Table 7.2).

Discussion on the estimation of likelihood and consequence in all groups of hazards used the scales ranging from “**negligible**” to “**very high**”. The value of each scale was calculated by considering the level of severity of the impacts of each hazard would have on humans, the coverage and duration of the impacts and the level of reversibility of each hazard.

Table 7.2 Scale and definition of each consequence that may result from each identified hazard

Scale	Definition
Negligible	Almost no change
Very low	Insignificant impact on human health and social economy
Low	Very low impact or no damage to the ecosystem
Moderate	Adverse health effects but is reversible and minor impact on the social economy Damage to the environment or disruption to local biodiversity that is reversible and limited in time and space or numbers affected
High	Adverse health effects that are difficult to reverse, but not life-threatening and lead to moderate social-economic impact Long term damage to the environment or disruption to biodiversity but is still reversible
Very high	Adverse health effects that are severe, widespread, irreversible, life-threatening and devastating to the social-economic conditions Extensive damage to the environment or extensive biodiversity and physical ecosystem, communities or an entire species that persist and is not readily reversible

After determining the values of the consequence for each hazard, the experts discussed where to place each hazard into a risk matrix (Table 7.3)

Table 7.3 Matrix of the level of Risk of each identified hazard

		Consequence					
Likelihood		Negligible	Very Low	Low	Moderate	High	Very High
	Negligible	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk	Very Low Risk
	Very Low	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk
	Low	Negligible Risk	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk
	Moderate	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	High Risk
	High	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk
	Very High	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk

The final elicitation was held among the core team only to synchronize all of the results into one final result against “cause more harm”. The Independent Team leader facilitated this final elicitation stage.

7.3. RESULTS OF SOLICITATION OF CONSEQUENCES

The expert team workshop reached a consensus on identified hazard. The solicitation indicated that the 57 hazards had moderate to high scales of consequence. The endpoint of the four groups were

dominated by moderate consequence (ecology, public health, and social-economy and culture) and high consequence (efficacy of mosquito management).

7.3.1. Ecology

The ecology component had the highest number of hazards (including endpoint). There were 19 hazards in this component, leading to 1 end point, which is ecological influence (Table 7.4). Experts solicitation indicated that the release of *Wolbachia*-infected *Ae. aegypti* in a particular area may lead to changes of the ecology of *Wolbachia*, vector and DENV of 74% (moderate consequence). Overall, hazards in the ecology component were estimated to have in average moderate consequence due to the ecological changes on both *Wolbachia*-infected *Ae. aegypti* and virus. 18 hazards in the ecology component, not including the endpoint, had been estimated to have moderate consequence (6 hazards), high consequence (7 hazards), and very high consequence (5 hazards) with between 57% to 90% consensus. Hazards of very high consequence were estimated to have negligible to very low likelihood.

7.3.2. Mosquito Management Efficacy

Expert solicitation of 12 hazards (including endpoint) in efficacy of mosquito management component resulted in a high consequence of 0.85 of the endpoint (Table 7.5). The other 11 hazards were widely estimated to have very low consequence (1 hazard), low consequence (2 hazards), moderate consequence (3 hazards), and high consequence (4 hazards).

7.3.3. Public Health

The 14 hazards in public health component were identified as a result of the release of *Wolbachia*-infected *Ae. aegypti*, leading to an endpoint of standard of public health with a value of consensus of 0.5 (moderate consequence) (Table 7.6). Expert solicitation of other 13 hazards indicated moderate consequence (4 hazards) and high consequence (9 hazards).

7.3.4. Socio-Cultural and Economic Impacts

The results of the consensus of the experts concluded that there were 13 hazards that may lead to economic, social, and cultural impact as a result of the release of *Wolbachia*-infected *Ae. aegypti*, with an endpoint of economic and socio-cultural impact (Table 7.7). Experts solicitation resulted in 0.5 (moderate consequence) of the endpoint of this component. Hazards in this component were calculated to have negligible consequence (5 hazards), very low consequence (1 hazard), low consequence (1 hazard), moderate consequence (2 hazards), and high consequence (3 hazards). Changes in the economy, health maintenance, tourism, decline in income and changes in expenditure were estimated to be negligible since they were calculated to have very low likelihood of occurrence.

7.4. RESULTS OF RISK ANALYSIS

The Risk Analysis workshop resulted in the consensus concerning the estimation of the likelihood and consequence of hazards. Both variables were combined to result in estimation of risks in the four components: Ecology, Efficacy of Mosquito Management, Public Health, and Economy and Socio Cultural, leading to endpoint risk of “cause more harm” as a result of the release of *Wolbachia*-infected *Ae. aegypti*.

7.4.1. Ecology

The first component of “cause more harm” was ecology. The release of *Wolbachia*-infected *Ae. aegypti* in a particular area may lead to adverse impacts to the ecology of the ecosystem of the area. There were 18 hazards in the component and one end point, that is the influence to the ecology (Table 7.4). The

results of the calculation of the probability of hazards indicated that 11 out of 18 hazards had negligible risk. Two hazards, namely “vector change” and “replacement of dengue vectors” were calculated as low risk hazards.

Table 7.4 Consensus on estimates of likelihood, consequence and risk in Ecology (ranked by severity of risk).

No	Ecology	Likelihood	Likelihood scale	Consensus on consequence	Scale of consequence	Consequence Risk	Risk matrix status
1	Ecological effect	0.0474	Very low	0.74	Moderate	0.035076	Negligible risk
2	Selection for more virulent arboviruses	0.01	Negligible	0.75	High	0.0075	Negligible risk
3	Invertebrate transfer and Wolbachia genome	0.001	Negligible	0.75	High	0.00075	Negligible risk
4	Change in genetic diversity	0.01	Negligible	0.74	Moderate	0.0074	Negligible risk
5	Increased host biting	0.01	Negligible	0.89	High	0.0089	Negligible risk
6	Female biased sex ratio	0.01	Negligible	0.57	Moderate	0.0057	Negligible risk
7	Increased filarial fitness	0.001	Negligible	0.75	High	0.00075	Negligible risk
8	Transfer of other arboviruses or parasites	0.001	Negligible	0.75	High	0.00075	Negligible risk
9	Mosquito host range	0.001	Negligible	0.74	Moderate	0.00074	Negligible risk
10	Ecosystem service change	0.001	Negligible	0.74	Moderate	0.00074	Negligible risk
11	Ecological niche	0.02	Very low	0.74	Moderate	0.0148	Negligible risk
12	Geographic distribution change	0.04	Very low	0.57	Moderate	0.0228	Negligible risk
13	Genetic biodiversity change	0.0063	Negligible	0.9	Very high	0.00567	Very low risk
14	Vertebrate transfer and Wolbachia genome	0.001	Negligible	0.95	Very high	0.00095	Very low risk
15	New mosquito species evolves	0.001	Negligible	0.95	Very high	0.00095	Very low risk
16	Density	0.05	Very low	0.75	High	0.0375	Very low risk
17	Environmental change	0.0114	Negligible	0.9	High	0.01026	Very low risk
18	Replacement of dengue vectors	0.05	Very low	0.9	Very high	0.045	Low risk
19	Vector change	0.102	Very low	0.9	Very high	0.0918	Low risk

Vector change is defined as the changes to density, behaviour, biology and reproduction of vector. One of the changes in behaviour as a result of the presence of *Wolbachia* is changes in blood feeding behaviour. Moreira *et al.* (2009b) reported that wMelPop-infected *Ae. aegypti* influenced blood-feeding behaviour among female adult *Ae. aegypti*. The older the *Wolbachia*-infected *Ae. aegypti*, the higher the intensity for blood-feeding. However, older mosquitoes spent more time in pre-probing and probing, in addition to shaking and bendy proboscis behaviour that lead to a decline in saliva production. This indicates that although the presence of wMelPop strain may cause an increase in blood feeding intensity

among female adults, the ability of the mosquitoes to find blood meals also declines. Accordingly, experts consider this risk negligible as it does not lead to any adverse impact.

Besides *Ae. aegypti*, other mosquito species such as *Ae. albopictus* (Higa 2011), *Ae. polynesiensis* (Rosen *et al.* 1954) and *Ae. scutellaris* (Moore *et al.* 2007) are also primary dengue vectors. Until now, *Ae. aegypti* are still the effective primary vector in transmission of DENV. History indicates that *Ae. aegypti* was first identified as the primary vector of yellow fever in 1648 in Mexico and Guadeloupe (Rogers *et al.* 2006). Record on the first epidemic of dengue fever transmitted by *Ae. aegypti* was first found to happen in 1779. Yellow fever started to become epidemic in the beginning of the 21st century while epidemic of dengue fever started in the 1950s. Both viruses belong to the Flaviridae virus family. However, they are never found at the same time in one particular endemic area. Based on this information, experts concluded that in the next 30 years there is a likelihood that there is a very low occurrence of vector replacement as a result of *Wolbachia*-infected *Ae. aegypti*.

Other hazard that was concluded to have negligible risk and moderate consequence was female biased sex-ratio. So far there has not been any report on the influence of *Wolbachia* to the sex ratio of *Ae. aegypti* mosquitoes or *Aedes* genus. However, Shaw *et al.* (2016) reported that infection of *Wolbachia* to the natural population of *Anopheles* had not influenced the sex ratio of the offspring. The result outlines the relatively low influence of *Wolbachia* to sex ratio of *Ae. aegypti* mosquitoes. To prove this, further in-depth exploration on the sex ratio of *Ae. aegypti* after being infected with *Wolbachia* need to be conducted.

During the formulation of the hazards, concerns arose on the possibility of the evolution of *Wolbachia* in *Ae. aegypti* that may lead to increased fitness of filarial nematodes in mosquitoes. The result of the consensus on this particular hazard indicated that there was a negligible risk of increased filarial fitness. Pfarr *et al.* (2007) concluded that *Wolbachia* that infect arthropods are distinct from *Wolbachia* that infect filarial nematodes. Pfarr explained that *Wolbachia* are parasites in arthropods but mutualists in filarial nematodes. In addition, arthropods and nematodes are originated from different phylum (Wang *et al.* 1999) that the risk of the evolution of *Wolbachia* present in *Ae. aegypti* in association with filarial nematodes is very low or very unlikely to happen.

7.4.2. Efficacy of Mosquito Management

One component of hazards that may occur as a result of the release of *Wolbachia*-infected *Ae. aegypti* is reduced efficacy of mosquito population management or control. Expert discussion indicated that there were 11 hazards leading to one endpoint, which is reduced efficacy of mosquito management. The consensus on estimation of risk to efficacy of mosquito management resulted in 7 hazards of negligible risk and 4 hazards of very low risk. Efficacy in mosquito management endpoint has a very low risk of 0.08925 with very low likelihood (0.105) and high consequence (0.85).

Table 7.5 Consensus on estimation of likelihood, consequence, and risk in Efficacy of Mosquito Management (ranked by level of severity of risks).

No	Efficacy of mosquito management	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
1	<i>Avoidance strategies</i>	0,05	<i>Very low</i>	0,1	<i>Very low</i>	0,005	<i>Negligible risk</i>
2	<i>Mosquito behaviour change</i>	0,1	<i>Very low</i>	0,7	<i>Moderate</i>	0,07	<i>Negligible risk</i>
3	<i>Insecticide resistance</i>	0,05	<i>Very low</i>	0,2	<i>Low</i>	0,01	<i>Negligible risk</i>
4	<i>Strain selection</i>	0,05	<i>Very low</i>	0,2	<i>Low</i>	0,01	<i>Negligible risk</i>
5	<i>Increased biting</i>	0,01	<i>Negligible</i>	0,8	<i>High</i>	0,008	<i>Negligible risk</i>
6	<i>Other pathogens</i>	0,0943	<i>Very low</i>	0,5	<i>Moderate</i>	0,04715	<i>Negligible risk</i>
7	<i>Mosquito management efficacy</i>	0,105	<i>Very low</i>	0,85	<i>High</i>	0,08925	<i>Very low risk</i>
8	<i>Increased complacency</i>	0,1	<i>Very low</i>	0,75	<i>High</i>	0,07	<i>Very low risk</i>
9	<i>Household control</i>	0,159	<i>Low</i>	0,6	<i>Moderate</i>	0,0954	<i>Very low risk</i>
10	<i>Increased difficulty to control</i>	0,0257	<i>Very low</i>	0,85	<i>High</i>	0,021845	<i>Very low risk</i>
11	<i>More dengue occurs</i>	0,0833	<i>Very low</i>	0,8	<i>High</i>	0,06664	<i>Very low risk</i>
12	<i>Increased dengue virulence</i>	0,04	<i>Very low</i>	0,8	<i>High</i>	0,032	<i>Very low risk</i>

There was a very low risk of the hazards identified in the public health component since most of the hazards were estimated to have low likelihood of occurrence. One of the hazards of very low risk was increased complacency as a result of successful control of mosquitoes. Chapter 6.3.2 explained about the very low likelihood of 10% that such risk may occur. However, this hazard was estimated to have a high consequence of 75%. It means that the hazard may have significant influence on the success in *Ae. aegypti* mosquito management. Successful community-based vector mosquito control is influenced by a number of factors, including community's alert of the distribution of mosquito population and the rate of virus transmission in their respective areas.

7.4.3. Public Health

The release of *Wolbachia*-infected *Ae. aegypti* did not affect public health because estimation of consequence indicated that there was a negligible risk (0.0348) with a scale of likelihood of 0.0696 and a scale of consequence of 0.5 (Table 7.6). Seven of 13 hazards have negligible risks.

Table 7.6 Consensus on estimation of likelihood, consequence and risk to Public Health (ranked by level of severity of risks).

No	Public health	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
1	Standard of public health	0.0696	Very low	0.5	Moderate	0.0348	Negligible risk
2	More cases	0.01	Negligible	0.8	High	0.008	Negligible risk
3	Severity of disease	0.01	Negligible	0.8	High	0.008	Negligible risk
4	Interference with other dengue controls	0.1	Very low	0.5	Moderate	0.05	Negligible risk
5	Feeding frequency	0.01	Negligible	0.75	High	0.0075	Negligible risk
6	Mosquito density	0.1	Very low	0.5	Moderate	0.05	Negligible risk
7	Increased biting	0.01	Negligible	0.8	High	0.008	Negligible risk
8	Other pathogens	0.0943	Very low	0.5	Moderate	0.04715	Negligible risk
9	Dengue evolution	0.05	Very low	0.85	High	0.0425	Very low risk
10	Dengue vector competence	0.05	Very low	0.8	High	0.04	Very low risk
11	Host preference	0.1	Very low	0.85	High	0.085	Very low risk
12	Non dengue vector competence	0.05	Very low	0.85	High	0.0425	Very low risk
13	Nuisance biting	0.146	Low	0.5	Moderate	0.073	Very low risk
14	Dengue transmission	0.151	Low	0.8	High	0.1208	Low risk

7.3.4. Economic and Socio Cultural Impact

The results of the consensus of the experts concluded that there were 12 hazards that may lead to economic, social, and cultural adverse impact to the community as a result of the presence of *Wolbachia*-infected *Ae. aegypti* (Table 7.7). The 12 hazards were calculated to have between negligible to moderate risks, which was dominated by negligible risk (7 hazards). Economic, social and cultural impact as a result of the presence of *Wolbachia*-infected *Ae. aegypti* was calculated to have a very low risk of 0.0915 with low likelihood (0.183) and moderate consequence (0.5).

Table 7.7 Consensus on estimation of likelihood, consequence and risk in economic, social, and cultural impact (ranked by level of severity of risks).

No	Economic and Socio-Cultural	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
1	Economic change	0.1	Very low	0.01	Negligible	0.001	Negligible risk
2	Health care	0.05	Very low	0.01	Negligible	0.0005	Negligible risk
3	Tourism	0.02	Very low	0.01	Negligible	0.0002	Negligible risk
4	Lost income	0.02	Very low	0.01	Negligible	0.0002	Negligible risk
5	Expense change	0.05	Very low	0.01	Negligible	0.0005	Negligible risk
6	Social-behavioural change	0.166	Low	0.2	Low	0.0332	Negligible risk
7	Migration	0.1	Very low	0.08	Very low	0.008	Negligible risk
8	Economic and socio-cultural effect	0.183	Low	0.5	Moderate	0.0915	Very low risk
9	Scapegoating	0.3	Low	0.45	Moderate	0.135	Very low risk
10	Adverse media	0.4	Low	0.75	High	0.3	Low risk
11	Social fear	0.5	Moderate	0.6	Moderate	0.3	Low risk
12	Class action	0.5	Moderate	0.75	High	0.375	Moderate risk
13	Social conflict	0.5	Moderate	0.75	High	0.375	Moderate risk

In the economic and socio-cultural component, experts identified hazards such as scapegoating, adverse media, social fear, class action and social conflict. Socio-cultural hazards were estimated to have higher risk than the economic ones. The hazards may likely to happen when information concerning technologies for controlling *Wolbachia* is not available in details and does not reach all elements of the society, who are the main actors in community based control.

7.5. SUMMARY

In general, the experts reached 57 consensus concerning estimation of the likelihood, consequence, and risk for “cause more harm” leading to one endpoint of “cause more harm”, 4 components of “cause more harm” namely ecological influence, efficacy of mosquito management, and economic and socio-cultural impact, and 52 other hazards (Table 7.8). The results of the expert solicitation of the 57 hazards that may occur as a result of the release of *Wolbachia*-infected *Ae. aegypti* indicated that there was an estimated high consequence of 0.8 of the endpoint for “cause ore harm” (Table 7.8). The consequence of the 56 hazards ranged from negligible (5 hazards), very low (3 hazards), low (3 hazards), moderate (17 hazards), high (23 hazards), and very high consequence (6 hazards). The hazards had consensus scores of between 1% to 95% with the scores for likelihood that were dominated by negligible likelihood.

Table 7.8 Summary of 57 consensus of estimation of likelihood, consequence and risk (ranked by risk) for “cause more harm” endpoint.

No	Node	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
1	Change in genetic diversity	0.01	Negligible	0.74	Moderate	0.0074	Negligible risk
2	Ecosystem service change	0.001	Negligible	0.74	Moderate	0.00074	Negligible risk
3	Female biased sex ratio	0.01	Negligible	0.57	Moderate	0.0057	Negligible risk
4	Mosquito host range	0.001	Negligible	0.74	Moderate	0.00074	Negligible risk
5	Feeding frequency	0.01	Negligible	0.75	High	0.0075	Negligible risk
6	Increased biting	0.01	Negligible	0.8	High	0.008	Negligible risk
7	Increased filarial fitness	0.001	Negligible	0.75	High	0.00075	Negligible risk
8	Increased host biting	0.01	Negligible	0.89	High	0.0089	Negligible risk
9	Invertebrate transfer and Wolbachia genome	0.001	Negligible	0.75	High	0.00075	Negligible risk
10	More cases	0.01	Negligible	0.8	High	0.008	Negligible risk
11	Selection for more virulent arboviruses	0.01	Negligible	0.75	High	0.0075	Negligible risk
12	Severity of disease	0.01	Negligible	0.8	High	0.008	Negligible risk
13	Transfer of other arboviruses or parasites	0.001	Negligible	0.75	High	0.00075	Negligible risk
14	Economic change	0.1	Very low	0.01	Negligible	0.001	Negligible risk
15	Expense change	0.05	Very low	0.01	Negligible	0.0005	Negligible risk
16	Health care	0.05	Very low	0.01	Negligible	0.0005	Negligible risk
17	Lost income	0.02	Very low	0.01	Negligible	0.0002	Negligible risk
18	Tourism	0.02	Very low	0.01	Negligible	0.0002	Negligible risk
19	Ecological effect	0.0474	Very low	0.74	Moderate	0.035076	Negligible risk
20	Avoidance strategies	0.05	Very low	0.1	Very low	0.005	Negligible risk
21	Migration	0.1	Very low	0.08	Very low	0.008	Negligible risk
22	Insecticide resistance	0.05	Very low	0.2	Low	0.01	Negligible risk
23	Strain selection	0.05	Very low	0.2	Low	0.01	Negligible risk
24	Ecological niche	0.02	Very low	0.74	Moderate	0.0148	Negligible risk
25	Geographic distribution change	0.04	Very low	0.57	Moderate	0.0228	Negligible risk

No	Node	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
26	Interference with other dengue controls	0.1	Very low	0.5	Moderate	0.05	Negligible risk
27	Mosquito behaviour change	0.1	Very low	0.7	Moderate	0.07	Negligible risk
28	Mosquito density	0.1	Very low	0.5	Moderate	0.05	Negligible risk
29	Other pathogens	0.0943	Very low	0.5	Moderate	0.04715	Negligible risk
30	Standard of public health	0.0696	Very low	0.5	Moderate	0.0348	Negligible risk
31	Social-behavioural change	0.166	Low	0.2	Low	0.0332	Negligible risk
32	Increased complacency	0.1	Very low	0.75	High	0.07	Very low risk
33	Environmental change	0.0114	Negligible	0.9	Very high	0.01026	Very low risk
34	Genetic biodiversity change	0.0063	Negligible	0.9	Very high	0.00567	Very low risk
35	New mosquito species evolves	0.001	Negligible	0.95	Very high	0.00095	Very low risk
36	Vertebrate transfer and Wolbachia genome	0.001	Negligible	0.95	Very high	0.00095	Very low risk
37	Dengue evolution	0.05	Very low	0.85	High	0.0425	Very low risk
38	Dengue vector competence	0.05	Very low	0.8	High	0.04	Very low risk
39	Density	0.05	Very low	0.75	High	0.0375	Very low risk
40	Host preference	0.1	Very low	0.85	High	0.085	Very low risk
41	Increased dengue virulence	0.04	Very low	0.8	High	0.032	Very low risk
42	Increased difficulty to control	0.0257	Very low	0.85	High	0.021845	Very low risk
43	More dengue occurs	0.0833	Very low	0.8	High	0.06664	Very low risk
44	Mosquito management efficacy	0.105	Very low	0.85	High	0.08925	Very low risk
45	Non dengue vector competence	0.05	Very low	0.85	High	0.0425	Very low risk
46	Economic and socio-cultural effect	0.183	Low	0.5	Moderate	0.0915	Very low risk
47	Household control	0.159	Low	0.6	Moderate	0.0954	Very low risk
48	Nuisance biting	0.146	Low	0.5	Moderate	0.073	Very low risk
49	Scapegoating	0.3	Low	0.45	Moderate	0.135	Very low risk
50	Replacement of dengue vectors	0.05	Very low	0.9	Very high	0.045	Low risk
51	Vector change	0.102	Very low	0.9	Very high	0.0918	Low risk

No	Node	Likelihood	Likelihood scale	Consequence consensus	Consequence scale	Consequence risk	Risk matrix state
52	Adverse media	0.4	Low	0.75	High	0.3	Low risk
53	Dengue transmission	0.151	Low	0.8	High	0.1208	Low risk
54	Social fear	0.5	Moderate	0.6	Moderate	0.3	Low risk
55	Class action	0.5	Moderate	0.75	High	0.375	Moderate risk
56	Social conflict	0.5	Moderate	0.75	High	0.375	Moderate risk
57	Cause More Harm	0.011	Negligible	0.8	High	0.0088	Negligible risk

Each consensus was afterwards grouped based on the risk matrix to get to the levels of severity of the risks between negligible risk (33 hazards), very low risk (17 hazards), low risk (5 hazards), and moderate risk (2 hazards). Among the four components of “cause more harm”, ecological influence and standard of public health were estimated to have negligible risk while efficacy of mosquito management and economic and socio-cultural impact components were estimated to have very low risk. Based on the estimation of the risk of 57 hazards for “**cause more harm**” endpoint, the release of *Wolbachia*-infected *Ae. aegypti* has negligible likelihood (0.011) and high consequence (0.8) that lead to **negligible risk** (0.0088).

Hazards that may occur as a result of the release of *Wolbachia*-infected *Ae. aegypti* were formulated in a matrix of risk estimation (Table 7.9). Each hazard was assigned with risk status using the value of likelihood and value of consequence in Table 7.8. Assigning each hazard in the matrix was done based on the scheme in Table 7.3.

Table 7.9 Matrix of risk estimation for “cause more harm” endpoint.

LIKELIHOOD	CONSEQUENCE				
	Negligible	Very low	Low	Moderate	High
Negligible	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk Change in genetic diversity Female biased sex ratio Mosquito host range Ecosystem service change	Negligible Risk Selection for more virulent arboviruses Invertebrate transfer and Wolbachia genome Increased host biting Increased filarial fitness Transfer of other arboviruses or parasites More cases Severity of disease Feeding frequency Increased biting Cause More Harm
					Very Low Risk Genetic biodiversity change Vertebrate transfer and Wolbachia genome New mosquito species evolves Environmental change
Very low	Negligible Risk Economic change Health care Tourism Lost income Expense change	Negligible Risk Migration Increased complacency Avoidance strategies	Negligible Risk Insecticide resistance Strain selection	Negligible Risk Ecological effect Ecological niche Geographic distribution change Mosquito behaviour change Standard of public health Interference with other dengue controls Mosquito density Other pathogens	Very Low Risk Density Mosquito management efficacy Increased difficulty to control More dengue occurs Increased dengue virulence Dengue evolution Dengue vector competence Host preference Non dengue vector competence
					Low Risk Vector change Replacement of dengue vectors
Low	Negligible Risk	Negligible Risk	Negligible Risk Social-behavioural change Nuisance biting	Very Low Risk Economic and socio-cultural effect Scapegoating Household control	Low Risk Adverse media Dengue transmission
					Moderate Risk
Moderate	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk Social fear	Moderate Risk Social conflict Class action
					High Risk
High	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	Extreme Risk
Very high	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	Extreme Risk

CHAPTER 8

CONSLUSIONS AND RECOMMENDATIONS

8.1. Conclusion

Aedes aegypti is a primary vector of dengue disease that has a cosmopolitan range, meaning that it is found in many cities around the globe. Thus far, mosquito disease vector control has been the most effective measure in addressing dengue disease. In Indonesia, measures have been promoted through the 3M (covering, draining, and burying unused water containers) program that has been considered effective in reducing mosquito population in the field. However, available data has indicated that incidence of dengue haemorrhage fever persists that pesticides are still commonly used as alternative measure in mosquito control in many locations in Indonesia.

The finding of *Wolbachia* is one of the breakthroughs due to its novelty and innovation in addressing problematic mosquito vector control. The decline in *Ae. aegypti* mosquito population due to Cytoplasmic Incompatibility (CI) and reduced vector competence are considered key in addressing problems associated with mosquito population, which has never been very successfully addressed. However, the novelty of the technology needs to be responded with caution as there are limited knowledge on the ecology of the *Wolbachia* itself. To this point, researches in a number of countries have indicated that *Wolbachia*-infected *Ae. aegypti* mosquitoes do not show any distinct behaviour compared with the wildtype population that are not infected by *Wolbachia*. However, the future is still beyond prediction and therefore a Risk Assessment was considered necessary to ensure that all potential adverse impacts can be anticipated.

The result of the Risk Assessment conducted in Indonesia has indicated that it is estimated that over the next 30 years, there would be a negligible risk of cause more harm as a result of the release of *Wolbachia*-infected *A. aegypti*

The results of the focus group discussion indicated a number of important feedbacks, including that continuous monitoring should be conducted of the release of *Wolbachia*-infected *Ae. aegypti* to prevent hazards identified in the assessment from happening in the natural environment.

In general, it can be concluded that all concerns concerning the release have emerged due to lack of current knowledge on *Wolbachia*. However, scientific data have so far been able to address the concerns that experts could reach to a consensus of the negligible risk. The expert team conducted risk analysis based on global evidence and expert judgement resulting from comprehensive experience in health entomology, evolution-ecology, public health, mosquito management, physiology, philosophy, economy, and social. Current available experiences are capable of covering all aspects of the release of *Wolbachia*-infected *Ae. aegypti* in an integrated manner that it can be said that the result of the assessment has completely covered all potential hazards. However, up to date knowledge should always be followed and taken into consideration for the program to be able to immediately respond to changes of hazard or potential increase of risk.

In this opportunity, we would also like to say that extreme caution must be taken in responding to the result of the Risk Assessment. Relatively high values have been assigned to the likelihood and

consequence of the identified hazards, especially in the economic and socio-cultural hazards of class action (likelihood: moderate, consequence: high, risk: moderate) and social conflict (likelihood: moderate, consequence: high, risk: moderate). The experts argued that both hazards are posing danger that high value have been assigned despite lack of scientific evidence that such hazards may occur. It indicates the high level of caution that the assessment has exercised.

8.2 Recommendations

Although the result of the Risk Assessment has provided the estimation that the likelihood and consequence of the hazards lead to negligible risk, the Expert Team has provided the following recommendations to the EDP Yogya Project:

- a. All government legislations concerning the permit for the release of *Wolbachia*-infected *Ae. aegypti* should be complied with. It has to be recognised, though, that the prevailing legislations do not have clear provisions with regard to the institutions and their corresponding roles and responsibilities, leading to confusion among project implementers. However, they should be complied with to ensure that no provisions and ethical principles are violated.
- b. The monitoring and evaluation (MONEV) plan of the EDP Project is very crucial in detecting possible risks that might ensue, and therefore a strong and rigorous Monitoring Plan should be an integral part of the project. A strong MONEV plan enables supervision as well as preparedness plan to anticipate unprecedented events and to ensure the project are capable to detect and be responsive to any possible risk that may arise in the future. The project should also make sure that local biosafety regulations and safety procedures are always complied with and referred to when undesirable events occur.

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