

**AN ECO-BIO-SOCIAL APPROACH  
TO ASSESS DENGUE TRANSMISSION DYNAMICS  
IN THAILAND**

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**A THESIS SUBMITTED IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY (BIOLOGY)  
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## AN ECO-BIO-SOCIAL APPROACH TO ASSESS DENGUE TRANSMISSION DYNAMICS IN THAILAND

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## ABSTRACT

This study integrates ecological, biological, and socio-demographic dimensions to assess dengue transmission dynamics. By employing two-stage random sampling, a total of 1,200 households (12 clusters; 100 each) situated in urban and semi-urban areas in 4 districts of the Chachoengsao Province were selected for cross-sectional longitudinal eco-bio-social interdisciplinary surveys. Cluster background, household, and entomological surveys, along with GIS mapping, were carried out by multidisciplinary teams during the rainy season, 2007 and the dry season, 2008. Laboratory mosquito blood meal analysis to assess dengue herd immunity in human populations was performed in 2009. Additionally, a stakeholder analysis was undertaken in 2010, so that the results could be triangulated with household and dengue vector survey data for contextualized eco-bio-social system analysis. In order to predict risks for *Aedes* vector abundance and dengue transmission, the study focused on four different ecotopes in urban and semi-urban areas: 1) Densely Populated Urban Residential Area or DENPURA 2) Commercial Area or C 3) Mixed Residential (with Commercial or RC) and 4) Mixed Residential (C plus DENPURA or RCDENPURA). Urban and semi-urban areas as predictors posed neither risks for an increase in vector density nor dengue transmission. In contrast, settings with high dengue endemicity corroborated a risk for dengue transmission but not for *Aedes* vector abundance, with RCDENPURA manifesting the highest risk. Entomological survey results revealed that 50-400L jars and cement bath basins were the two most productive container types. These key containers were significantly associated with their locations (inside the house and shaded area) and household usage (tap water and without intervention/cover). RC possessed the highest number of pupae per person (PP) in both seasons. The highest PPs were found in RCDENPURA during the wet season and DENPURA during the dry season. Mosquito blood meal analysis showed that the percentage of DENV IgM and IgG positive blood meals did not significantly differ between urban and semi-urban localities, but they were significantly associated with the degree of transmission. The highest risk for active dengue transmission was found in DENPURA during the wet season and RCDENPURA during the dry season. A seasonal pattern of DENV IgM and IgG seroprevalences demonstrated higher seropositivity rates during the dry season than the wet season. When blood meal analysis was linked with actual transmission, the number of positive DENV IgM correlated well with the number of reported dengue cases during the same period. This study is the first time that blood meal has been used to compare active dengue transmission among ecotopes. Social science research in relation to quantitative socio-demographic parameters showed housing structures with concrete/stone/brick in conjunction with improper garbage management and poor water storage practices as possible causes of increased vector density and dengue transmission, while “movement during the last 3 months” and “years of schooling longer than 5 years” also played a role in dengue transmission, especially in RCDENPURA. Stakeholder qualitative analysis, in light of their functions and interests, provided much more insight into the vector control problems and the persistence of dengue in the study area. In summary, a great variety of eco-bio-social determinants should be taken into consideration when formulating effective local dengue prevention/control programs.

KEY WORDS: DENGUE/ECOTOPE/BLOOD MEAL ANALYSIS/DENGUE HERD IMMUNITY/  
ECO-BIO-SOCIAL INTERDISCIPLINARY APPROACH

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การใช้วิธีการทางด้านนิเวศ-ชีวะ-สังคมในการประเมินพลวัตการแพร่เชื้อของโรคไข้เลือดออกในประเทศไทย  
 AN ECO-BIO-SOCIAL APPROACH TO ASSESS DENGUE TRANSMISSION DYNAMICS IN THAILAND

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บทคัดย่อ

การศึกษานี้ผสมผสานความรู้ด้านนิเวศวิทยา ชีววิทยาและสังคมประชากร เพื่อประเมินพลวัตการแพร่เชื้อของโรคไข้เลือดออก กลุ่มบ้านทั้งหมด 1,200 หลังคาเรือน (หรือ 12 กลุ่มบ้านๆ ละ 100 หลังคาเรือน) ซึ่งกระจายอยู่ในเขตเมืองและเขตกึ่งเมืองในพื้นที่ 4 อำเภอของจังหวัดฉะเชิงเทรา ได้ถูกคัดเลือกโดยวิธีสุ่มสองชั้น เพื่อสำรวจแบบบูรณาการสหสาขาวิชานิเวศ-ชีวะ-สังคม การสำรวจภูมิหลังของกลุ่มบ้าน ครวเรือน และกิจกรรมรวมถึงการทำแผนที่ GIS ได้ดำเนินการในช่วงฤดูฝนปี พ.ศ. 2550 และในช่วงฤดูร้อนปี พ.ศ. 2551 ตัวอย่างยุงตัวเต็มวัยที่เก็บจากทั้งสองฤดู ได้รับการวิเคราะห์ทางห้องปฏิบัติการเพื่อประเมินภาวะภูมิคุ้มกันของประชากรคนต่อการติดเชื้อไวรัสไข้เลือดออก ในปี พ.ศ. 2552 นอกจากนี้ การวิเคราะห์ผู้มีส่วนได้ส่วนเสียได้ดำเนินการ ในปี พ.ศ. 2553 เพื่อนำผลการวิเคราะห์ไปผนวกกับข้อมูลจากการสำรวจครวเรือนและชีววิทยา สำหรับการประเมินความเสี่ยงต่อการเกิดโรคในบริบทของระบบนิเวศ-ชีวะ-สังคม ได้ศึกษาความชุกชุมของยุงลายพาหะและการแพร่ระบาดของโรคไข้เลือดออก โดยแยกตาม ecotope 4 ลักษณะที่แตกต่างกัน ซึ่งกระจายอยู่ในเขตเมืองและกึ่งเมือง ได้แก่ [1] Densely Populated Urban Residential Area หรือ DENPURA 2) Commercial Area หรือ C 3) Mixed Residential (with Commercial หรือ RC) และ 4) Mixed Residential (C plus DENPURA หรือ RC DENPURA) พื้นที่เขตเมืองและเขตกึ่งเมืองซึ่งเป็นปัจจัยคัดทำนาย ไม่บ่งชี้ถึงความเสี่ยงต่อความชุกชุมของยุงลายพาหะและการแพร่ระบาดของโรคที่แตกต่างกัน ในทางตรงกันข้าม พื้นที่แคว้นเชื้อไข้เลือดออกสูงเสี่ยงต่อการแพร่ระบาดของโรคไข้เลือดออกแต่ไม่เสี่ยงต่อความชุกชุมของยุงลายพาหะ RC DENPURA เป็น ecotope ที่มีความเสี่ยงสูงที่สุดต่อการแพร่ของโรคไข้เลือดออก ผลการสำรวจทางชีววิทยาพบว่า ภาชนะกักเก็บน้ำที่มีการสำรวจพบลูกน้ำมากที่สุดคือ โอ่งน้ำ ขนาด 50-400 ลิตร และถังซีเมนต์ในห้องน้ำ ภาชนะ 2 ชนิดนี้มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับตำแหน่งที่วางภายในบ้านและตำแหน่งที่อยู่ใต้ร่มเงา เช่นเดียวกับกับการใช้น้ำในครวเรือนที่เป็นน้ำประปาและการไม่ใช้มาตรการควบคุมลูกน้ำและปิดฝาโอ่ง RC เป็น ecotope ที่พบจำนวนตัวไม่ต้อคน (Pupae per Person หรือ PP) สูงที่สุดทั้งสองฤดู RC DENPURA เป็น ecotope ที่พบดัชนี PP สูงที่สุดในฤดูฝน DENPURA เป็น ecotope ที่พบดัชนี PP สูงที่สุดในฤดูร้อน การวิเคราะห์ตัวอย่างเลือดคนจากยุงที่มีเลือดในท้อง พบว่าตัวอย่างเลือดที่มีผลบวกต่อ DENV IgM และ IgG ทั้ง 2 ฤดู ไม่มีความแตกต่างระหว่างพื้นที่เขตเมืองและเขตกึ่งเมือง แต่กลับมีความสัมพันธ์อย่างมีนัยสำคัญกับพื้นที่ที่มีการระบาดของโรคสูงในช่วงฤดูฝน พบตัวอย่างเลือดที่มีผลบวกต่อ IgM สูงสุด ใน ecotope แบบ DENPURA ในขณะที่ในฤดูร้อน พบตัวอย่างเลือดที่มีผลบวกต่อ IgM สูงสุด ใน ecotope แบบ RC DENPURA รูปแบบการกระจายของตัวอย่างเลือดคนที่มีผลบวกต่อ DENV IgM และ IgG ตามฤดูกาล แสดงให้เห็นว่าฤดูร้อนมีตัวอย่างเลือดคนที่มีผลบวกสูงกว่าฤดูฝน เมื่อวิเคราะห์เชื่อมโยงกับการแพร่ โรคตามธรรมชาติ พบว่าจำนวนตัวอย่างเลือดที่มีผลบวกด้วย DENV IgM สัมพันธ์กับจำนวนผู้ป่วยโรคไข้เลือดออกในช่วงระยะเวลาเดียวกัน การศึกษานี้ นับว่าเป็นครั้งแรกในการประเมินภาวะการระบาดของโรคไข้เลือดออกโดยใช้ตัวอย่างเลือดคนจากห้องยุง การวิจัยสังคมศาสตร์ที่เกี่ยวข้องกับปัจจัยทางประชากรสังคมในเชิงปริมาณ ซึ่งเห็นว่าสภาพโครงสร้างบ้านเรือนที่มีการก่อสร้างด้วยอิฐถือปูน การจัดการขยะมูลฝอยและการเก็บกักน้ำใช้ที่ไม่เหมาะสม เป็นสาเหตุที่ก่อให้เกิดการเพิ่มจำนวนของยุงลายพาหะ ซึ่งเพิ่มโอกาสเสี่ยงต่อการระบาดของโรคไข้เลือดออก นอกจากนี้ปัจจัยเรื่องการเคลื่อนย้ายของประชากรในช่วง 3 เดือนที่ผ่านมาและระยะเวลาการศึกษาเกินกว่า 15 ปี ก็เป็นปัจจัยสำคัญในการระบาดของโรค โดยเฉพาะอย่างยิ่ง ในพื้นที่ ecotope แบบ RC DENPURA การวิเคราะห์ผู้มีส่วนได้ส่วนเสียในเชิงคุณภาพเกี่ยวกับบทบทน์ ที่และคนสนใจ ก่อให้เกิดความเข้าใจอย่างล่องเท้าของปัญหาที่เกี่ยวกับการควบคุมยุงพาหะนำโรคไข้เลือดออกและการปรากฏของโรคอย่างต่อเนื่องในพื้นที่ศึกษา โดยสรุปการกำหนดแผนการป้องกันควบคุมโรคไข้เลือดออกที่มีประสิทธิภาพในระดับท้องถิ่น ควรจะต้องคำนึงถึงความแตกต่างของปัจจัยทางนิเวศ-ชีวะ-สังคมดังกล่าวข้างต้น

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## LIST OF ABBREVIATIONS

BI	Breteau index
C	Commercial area
CH	Community Hospital
CI	Container index
95%CI	95% confidence interval
CL	Community leader
CLIP	Collaboration and Conflict, Legitimacy, Interests, Power
CVVD	Center of Excellence for Vectors and Vector-Borne Diseases
DENPURA	Densely populated urban residential area
DENV	Dengue virus serotypes 1-4
DENV1	Dengue virus serotype 1
DENV2	Dengue virus serotype 2
DENV3	Dengue virus serotype 3
DENV4	Dengue virus serotype 4
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DPHE	Division of Public Health and Environment
DPHO	District Public Health Office
DSS	Dengue shock syndrome
ELISA	Enzyme linked immunosorbent assay
GH	General Hospital
GIS	Geographic Information System
GMC	Garbage management company
GPS	Global Positioning System
HC	Health Center
HI	House index
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid

## LIST OF ABBREVIATIONS (cont.)

IgG	Immunoglobulin G
IgM	Immunoglobulin M
L	Liter
OD	Optical density
OPD	o-phenyldiamine dihydrochloride solution
OR	Odds ratios
PAO	Provincial Administrative Organization
PBS	Phosphate-buffered saline solution
PBT	Phosphate-buffered saline solution with 0.5% casein and 0.05% Tween-20
PH	Private Hospital
PP	Pupae per person
PPHO	Provincial Public Health Office
PWUP	Public Works and Urban Planning
RC	Residential mixed with commercial area
RCDENPURA	Residential mixed with commercial area and densely populated urban residential area
$R_o$	The reproductive rate
SES	Socio-economic status
SWD	Sanitary Works Division
WSC	Water Supply Company
WSG	Waterworks Division/ Waterworks Authority

## CHAPTER I

### INTRODUCTON

Dengue is a spectrum of mosquito-borne disease caused by any one of four closely-related, but antigenically-distinct, dengue virus serotypes (DENV1-4) affecting humans. Any of the four dengue virus infections may become asymptomatic or may result in undifferentiated fever, dengue fever (DF), or dengue haemorrhagic fever (DHF) with plasma leakage that could lead to hypovolemic shock, known as dengue shock syndrome (DSS). DF and DHF remain principally infectious diseases of tropical and subtropical regions, and the four dengue serotypes are capable of maintaining a cycle that involves humans and the *Aedes* mosquito. Two competent mosquito vectors include *Aedes aegypti* and *Ae. albopictus*, yet *Ae. aegypti* (which is a domestic, day-biting mosquito that prefers to feed on humans) has become the most common *Aedes* species (Centers for Disease Control and Prevention, 2009; Cook, Zumla, & Manson, 2009).

Major epidemics of a sickness comparable to DF were first documented as occurring in Asia, Africa, and North America in 1779 and 1780. These disease pandemics happened every 10-30 years until World War II (1939-45). Since then infrequent epidemic occurrences have continued throughout tropic and sub-tropic regions. Soon after World War II, the first reported epidemic of DHF occurred in Manila, Philippines in 1953 and 1954, followed by epidemics in Bangkok, Thailand in 1958 and Malaysia, Singapore, and Vietnam in the 1960s. Because of the economic boom and rapid urbanization that occurred in Southeast Asia during the post-war period, DF and DHF epidemics expanded throughout the entire region during the 1970s.

In the Americas, after a long period of absence, many countries began encountering great epidemics of dengue during the 1980s due to the earlier disbandment of the *Ae. aegypti* eradication program. Not long after the 1980s resurgence in the American region, both the mosquito vectors and dengue viruses spatially distributed throughout the tropics (Gratz & Knudsen, 1997; Gubler, 1998b; Thongcharoen, 1993). Nowadays, it is

evident that dengue is one of the most serious communicable tropical diseases, placing more than 2.5 billion people at risk for infections, resulting in tens of million cases of DF, as well as hundreds of thousands of cases of DHF, each year (World Health Organization, 2009).

DHF was first recorded in Thailand in 1950, but the first official report occurred in 1958, with 2,500 cases reported in the Bangkok metropolitan area. The case numbers from 1958 to 1987 show a gradual tendency of increase (Ungchusak & Kunasol, 1988; Wellmer, 1983). During the initial period of outbreaks, most of the cases emerged in Bangkok and the surrounding area. But since 1965, dengue incidence has been widely spread throughout the whole country (Rojanapithayakorn, 1998). Some different patterns of disease outbreaks during the past 5 decades can be seen by examining related data over ten-year periods (Cummings et al., 2004; Sucharit, 1993).

Due to the lack of a competent tetravalent dengue vaccine that can secure life-long immunization, *Aedes* mosquito control measures have primarily been employed in the forms of primary prevention and disease outbreak interruption (World Health Organization, 1995). The prevention and control of dengue involve incalculable socio-cultural and operational problems (Gubler & Kuno, 1997). The few successful examples of vector control through community participation or in an aggregate combination method are unlikely sustainable (Kay & Vu, 2005; Kittayapong, Yoksan, Chansang, Chansang, & Bhumiratana, 2008; Ooi, Goh, & Gubler, 2006). Globally, dengue incidence to date fluctuates cyclically in space and time and never absolutely disappears from its originated transmission areas. As a result, each nation shouldering a dengue burden continually attempts diverse control efforts with the hope of establishing sustainable measures for combating the disease. Meanwhile, various research efforts have successively been conducted globally in order to facilitate new control measures. In addition, the World Health Organization established the global strategy for prevention and control of DF/DHF in 1995 with the goal of suppressing the dengue disease burden worldwide (World Health Organization, 1999b).

In reality, DF and DHF have a very complex epidemiology resulting from the underlying biotic and abiotic determinants that are responsible for interactions between human and natural systems. More specifically, several factors are believed to impact the rise and fall of this disease, including environmental and climate factors, predator-prey dynamics between the pathogen and host population, and viral factors.

However, certain underlying factors are only associated with the more severe forms of the dengue virus; other causes remain the subject of debate and speculation. Regarded as reemerging infectious diseases (Gratz & Knudsen, 1997; Gubler, 1998b, 2002; Kyle & Harris, 2008; Wilcox & Colwell, 2005), DF and DHF reflect the failure of prevention and control efforts, despite the fact that some success cases of vector control in the Americas, Cuba, and Singapore seem to have shortened the outbreak period or stopped it from spreading (Brightmer & Fantato, 1998; G. P. Kouri, Guzman, Bravo, & Triana, 1989; Tan, 1997). Although the determinants responsible for the reemergence of DF and DHF are not completely understood, socio-demographic parameters, the dynamics of the virus in vector- and human-host populations, and changeable health systems appear to play pivotal roles in this disease's resurgence.

Research attempts relevant to ecological and environmental determinants have been conducted to examine their impact on dengue emergence or reemergence. With regards to ecological factors that contribute to dengue transmission, research findings have shown that the dengue virus, host populations, and ecosystems interact within complex systems (Bonet et al., 2007; Despommier, Ellis, & Wilcox, 2006; Horwitz & Wilcox, 2005; Koopman & Longini, 1994). Other investigations related to environmental and climatological factors have revealed that temperatures and nutrients could affect the growth development and survival of *Aedes* larvae, shorten the extrinsic incubation period of the dengue virus, and increase the incidence of DHF, while an increase of rainfall could cause a decrease of incidence (Johansson, Cummings, & Glass, 2009; Jury, 2008; Su, 2008; Thammapalo, Chongsuwiatwong, McNeil, & Geater, 2005; Tun-Lin, Burkot, & Kay, 2000; Watts, Burke, Harrison, Whitmire, & Nisalak, 1987; Wiwanitkit, 2006).

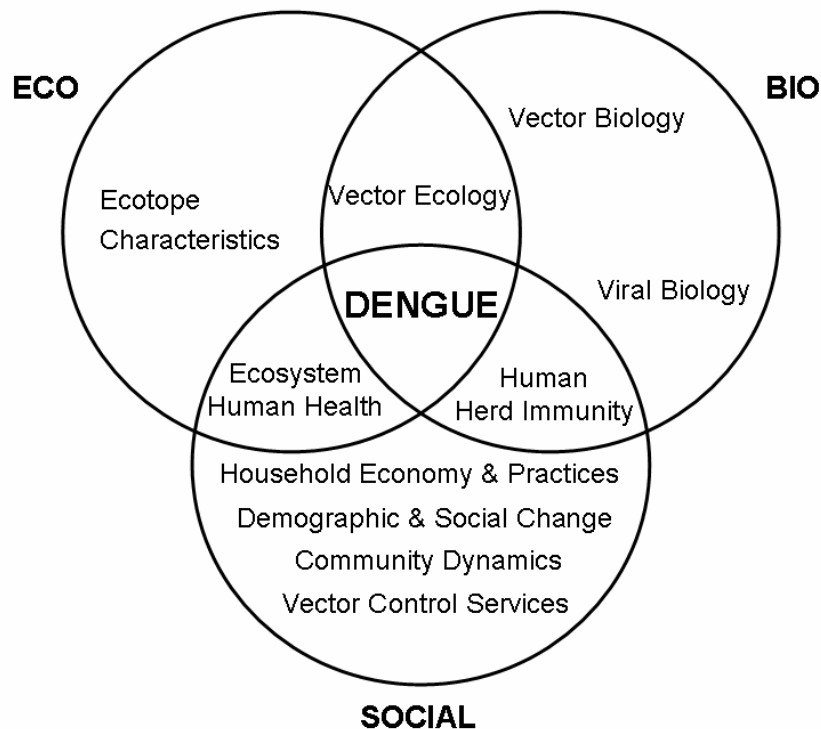
Research into the biology of the *Aedes* vector, dengue virus, and human host also have significant implications on DF/DHF prevention and control. A variety of research attempts involving vector biology, abundance, and distribution have provided better understanding of their correspondence with dengue virus circulation and disease transmission (Leishnam & Juliano, 2009; Maciel-de-Freitas, Peres, Souza-Santos, & Lourenco-de-Oliveira, 2008; Strickman & Kittayapong, 2003; Troyo et al., 2008; Tun-Lin, Kay, Barnes, & Forsyth, 1996). To get an insight into the dynamics of the

dengue virus and its human host populations for vaccine development or interrupting transmission, these need to look into genetic epidemiology, evolutionary biology, clinical pathology, immunology, underlying disease occurrence, and virulence (Endy, Chunsuttiwat et al., 2002; Endy, Nisalak et al., 2002; Garcia-Rejon et al., 2008; Mammen et al., 2008; Schreiber et al., 2009; Wearing & Rohani, 2006).

In addition to the eco-bio aspects mentioned above, socio-demographic approaches are needed to design effective and sustainable DF/DHF prevention and control measures. Using both qualitative and quantitative tools to understand socio-demographic parameters, scientists can work towards figuring out what determinants result in an increased vulnerability to DF/DHF. In some cases, the application of appropriate vector control measures coupled with community participation have been demonstrated to be more effective and sustainable than anti-mosquito approaches alone (Butraporn, Saelim, & Sitaputra, 1999; Kay & Vu, 2005; Kittayapong et al., 2008; Lok, 1985; Perez et al., 2007; Toledo et al., 2008). Additionally, several findings have concluded that there is a poor correlation between knowledge and behavioral change; thus control intervention programs have shifted to those that not only involve behavioral change but also those that take into account rapidly growing urbanization factors, such as modern transportation, high population growth, and societal changes (Callaway, 2007; Guha-Sapir & Schimmer, 2005; Melo et al., 2007; Schreiber et al., 2009; Sornmani, Okanurak, & Indaratna, 1995; Whiteford, 1997; World Health Organization, 1999b).

Although various research attempts have provided diverse lines of evidence about the drivers of vector abundance, virus circulation, and dengue transmission, all have been individually focused. Such an individual approach is no longer considered effective for figuring out the complexity of factors underlying the three epidemiological components at play. Theoretically, comprehensive DF/DHF management requires getting an insight into the holistic disciplines that can assess the driving determinants and how they significantly interact. The need for interdisciplinary integration of ecological, biological, and socio-demographic dimensions of dengue has been recently confirmed by dengue experts in different disciplines (Bonet et al., 2007; Daszak, 2005; Ellis & Wilcox, 2009; Holling, 2001; Ooi & Gubler, 2009; Spiegel et al., 2005; Wilcox & Colwell, 2005).

Therefore, our study applied a holistic transdisciplinary eco-bio-social conceptual framework (Arunachalam et al., 2010) to inquire what parameters of the three main groups play a significant role in three major consequences: *Aedes* mosquito abundance, dengue transmission, and dengue virus circulation and/or human herd immunity (Figure 1). Moreover, the ecosystem concept that focuses on ecotope characterization and associated factors as predictors for dengue transmission was employed because more gradient urbanization and population growth results in more complex and dynamic socio-demography of the inhabitants living in urban and semi-urban settings (Spiegel et al., 2004; Strickman, Sithiprasasna, Kittayapong, & Innis, 2000; Teixeira Mda et al., 2002; Wu et al., 2009). Lastly, this study is the first time that blood meal has been used to compare active dengue transmission among ecotopes.



**Figure 1** Conceptual framework of the study.

Adapted from the conceptual framework of the multi-country study on eco-bio-social research on dengue in Asia (Arunachalam et al 2010)

## 1.1 Research Outcomes

Based upon the significance of empirically important findings, this study expects not only to provide insight into the interdisciplinary eco-bio-social determinants responsible for triad interplay among influencing dengue vectors, vulnerable host populations, and the affecting virus that gives rise to dengue transmission or outbreak but into ways that more appropriately effective and sustainable dengue control measures can be applied to or used in diverse-ecotoped urban and semi-urban settings. We also expect our findings to enhance the application of development tools to be used in different dengue endemic areas.

## 1.2 Study Objectives

1. Examine four types of ecotope (densely populated urban residential area (DENPURA), commercial area (C), and mixed residential (R): commercial plus DENPURA or commercial alone) as drivers contributing to dengue vector abundance and the emergence of dengue in human hosts.
2. Evaluate dengue vector abundance and relevant environmental factors in the wet and dry seasons in terms of container types, their social use, and productivity.
3. Compare dengue herd immunity of human population (using mosquito blood meal) between the wet and dry seasons, among four different ecotopes belonging to urban and semi-urban settings, as well as between in high and low dengue transmission areas.
4. Determine socio-economic-demographic characteristics favoring vector density and dengue occurrence among the ecotopes of studied localities and between high and low dengue transmission settings.

## **CHAPTER II**

### **LITERATURE REVIEW**

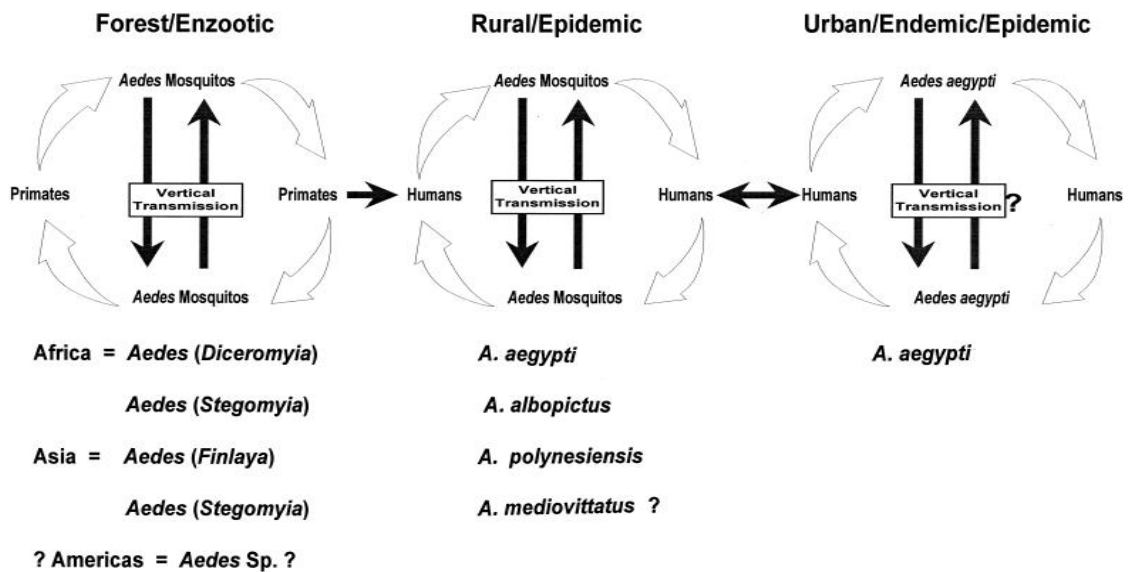
#### **2.1. General Overview**

##### **2.1.1 The dengue virus**

Dengue virus belongs to the genus *Flavivirus* and family *Flaviviridae*. There are four closely-related, but antigenically-distinct, dengue virus serotypes (DENV1-4). Using molecular clock techniques, it is estimated that the present DENV serogroup ancestor emerged about 1,000 years ago (Twiddy, Holmes, & Rambaut, 2003). Most phylogenies demonstrate that DENV4 is the most divergent serotype, followed by DENV2, and then DENV1 and DENV3 as the most closely related serotypes (Lewis et al., 1993; Twiddy et al., 2003; Zanotto, Gould, Gao, Harvey, & Holmes, 1996). These relatively small (40-50 nm) viruses contain single-stranded RNA. The virion is composed of a nucleocapsid with cubic symmetry enclosed in a lipoprotein envelope. Its genome is approximately 11,000 base pairs long, and comprises three structural protein genes encoding the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E), and seven nonstructural protein genes. The envelope glycoprotein is responsible for viral haemagglutination and neutralization activity (World Health Organization., 1997).

There are three main complexes within the family *Flaviviridae*, including tick-borne encephalitis virus, Japanese encephalitis virus, and dengue virus. All flaviviruses contain common group epitopes on the envelope protein that give rise to extensive cross reactions in serological tests. Consequently, these techniques may not be specific enough to distinguish the virus species among these virus groups. Given a distinct complex of antigenic and biological traits among four dengue serotypes, infection with any one serotype provides lifelong immunity to that virus serotype, but the other three serotypes elicit cross-protection for only a few months. Hence, persons living in a dengue endemic area can be infected with three to four dengue serotypes during their lifetime (Cook et al., 2009; Gubler, 1988; Hotta, 1969).

The transmission cycles of dengue viruses (Figure 2) can be divided into three types: 1) the forest/enzootic transmission cycle, 2) the rural/epidemic transmission cycle, and 3) the urban/endemic/epidemic transmission cycle (Gubler, 1988). The primitive enzootic transmission cycle of dengue viruses implicates canopy-inhabiting *Aedes* mosquitoes and lower primates in the rain forests of Asia and Africa. Present findings indicate that these viruses translocate irregularly from a forest origin to urban locations. An epidemic transmission cycle is thought to happen in rural villages or islands, where the human population is small. It is in these areas that the majority of susceptible inhabitants are swiftly infected by introduced viruses. The most significant transmission cycle with regards to public health burden is the urban endemic/epidemic cycle in large urban settings of the tropical zone. An *Ae. aegypti*-human-*Ae. aegypti* cycle maintains the circulation of viruses with periodic epidemics. The cocirculation of multiple virus serotypes in a particular area is considered hyperendemicity.



**Figure 2** Transmission cycles of dengue viruses.  
 (Gubler, 1998, p.485)

### 2.1.2 The vector

*Aedes* mosquitoes, the vector that transmits dengue viruses to humans, belong to the subgenus *Stegomyia*. *Ae. aegypti* is the principal vector, while other species such as *Ae. albopictus*, *Ae. polynesiensis*, members of *Ae. scutellaris*, and *Ae.*

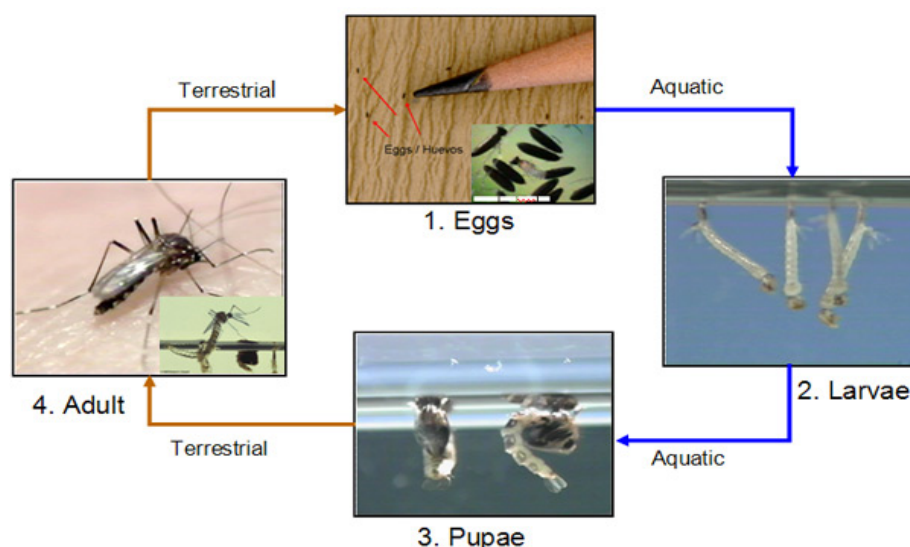
(*Finlaya niveus*) have been incriminated as secondary vectors. All except *Ae. Aegypti* exhibit their own limited spatial distribution, and although these other species appear to be excellent hosts for dengue viruses, they ordinarily remain less efficient epidemic vectors than *Ae. Aegypti* (World Health Organization, 1999a). It is thought that *Ae. Aegypti* originated in the tropical forests of Africa and likely spread throughout the rest of the globe via slave and trading ships during the seventeenth to nineteenth centuries (Gubler & Kuno, 1997; Smith, 1956). A number of forest-dwelling *Aedes* mosquitoes, known as tree-hole mosquitoes, have been determined as vectors of a sylvatic cycle of DENV involving mainly nonhuman primates in the forests of Africa (DENV2) and Southeast Asia (DENV1-4). The viruses in this sylvatic cycle are phylogenetically distinct from those in the urban cycle of dengue involving *Ae. Aegypti* (Wang et al., 2000). DENV is believed to have emerged from the jungles of Southeast Asia, with *Ae. albopictus* or possibly other *Aedes* species maintaining the virus in a sylvatic cycle involving nonhuman primates and humans residing in the countryside.

*Aedes* mosquitoes have a complex life-cycle (Figure 3) with dramatic changes in shape, function, and habitat (Centers for Disease Control and Prevention, 2009). Female mosquitoes lay their eggs on damp surfaces in water-holding containers. Eggs are deposited singly on wet walls just above the water line. Larvae hatch once the containers are flooded. The larvae pass through four developmental stages, the duration of which depends on the temperature, availability of food, and larval density in the receptacle. If the fourth instar has completely developed, metamorphosis is triggered, changing the larva into a pupa. Pupae do not feed but just change in form until the body of adult mosquito is formed. The whole life cycle takes approximately 8-10 days at room temperature. Hence, the life cycle of *Aedes* mosquitoes requires both aquatic (larvae, pupae) and terrestrial phases (eggs, adults). Soon after emergence, the adult mosquitoes mate and the inseminated female may take a blood meal within 24-36 hours. Blood is the source of protein essential for the maturation of eggs. This requirement of mosquito survival enables the dengue viruses to readily complete their transmission cycle. It is only the *Aedes* female mosquitoes that transmit the dengue viruses to human hosts. After they bite an infected person during the febrile viremic phase, those mosquitoes may get infected and undergo

an extrinsic incubation period of 8 to 12 days. Subsequently, they may transmit these viruses to uninfected persons.

*Ae. aegypti* is highly anthropophilic, although it may take meals from other available warm blooded animals. Being a diurnal species, females have two periods of biting activity, one in the morning for several hours after sunrise and the other in the afternoon for several hours before sunset. The female mosquitoes are very nervous feeders. When the feeding process is disrupted, they may feed on more than one person. It is this behavior that makes *Ae. aegypti* such an efficient epidemic vector. *Ae. aegypti* prefers to rest in dark, humid, secluded places inside houses or buildings, and the flight range of the adult female appears to be often limited to within 100 meters of the site of emergence.

*Ae. albopictus* is primarily a forest species that has become adapted to rural, suburban, and urban human environments. It generally oviposits and develops in natural containers such as tree holes, bamboo stumps, and leaf axils. In urban settings, however, this species may occupy either natural or artificial containers as a breeding site. *Ae. albopictus* is an indiscriminate blood taker and more zoophilic than *Ae. aegypti*. Its flight range may be up to 500 meters. Unlike *Ae. aegypti*, some strains are cold adapted in Northern Asia and America, with eggs that spend the winter in diapause. In the laboratory, both species can transmit dengue virus vertically from a female to eggs to her offspring, although *Ae. albopictus* does so more readily.



**Figure 3** The life cycle of *Aedes* mosquitoes.

Image obtained from <http://www.cdc.gov/Dengue/entomologyEcology/index.html>

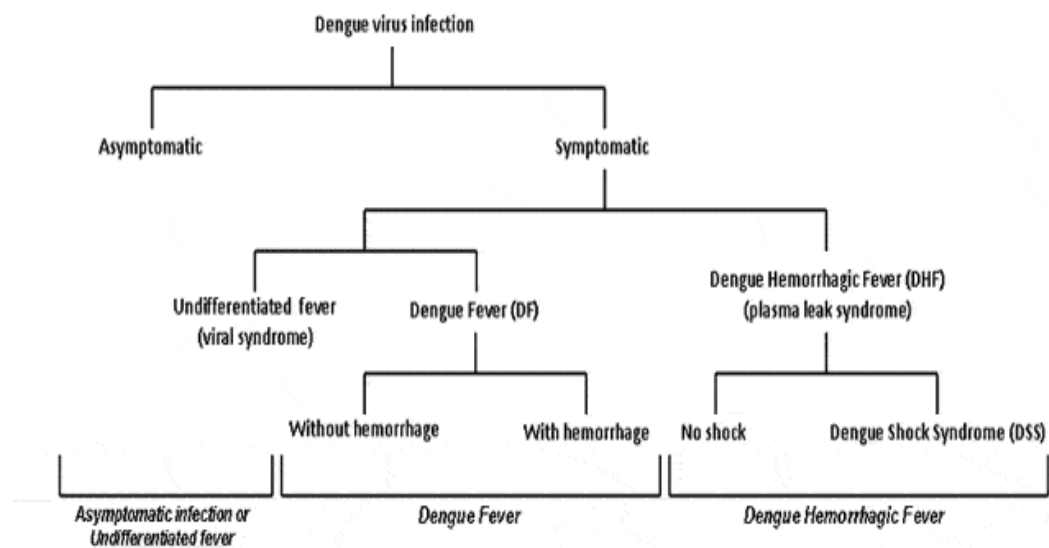
### 2.1.3 The human host

The human host is one of the three essential components (human, mosquito, and virus) required for dengue transmission to occur. After a person is bitten by an infected *Aedes* female mosquito, the virus undergoes an incubation period of 3 to 14 days, after which the person may be asymptomatic or experience fever, dengue fever (DF), or dengue haemorrhagic fever (DHF) with plasma leakage that may give rise to hypovolemic shock, known as dengue shock syndrome (DSS) (Figure 4) (Centers for Disease Control and Prevention, 1986; World Health Organization, 2009). DF is an acute febrile illness of 2-7 days duration with two or more clinical manifestations, including headache, retro-orbital pain, myalgia, arthralgia, and rash. DHF is defined as an acute febrile illness with minor or major bleeding, and its haemorrhagic tendency is marked by increased vascular permeability (plasma leakage), thrombocytopenia, and haemorrhagic manifestations. DSS results when fluid leakage into the interstitial spaces causes hypovolemic shock, which without appropriate treatment may lead to death.

Obvious disease manifestations that result from dengue have been depicted as just the tip of the iceberg (Kurane & Ennis, 1992). Less than 10% of symptomatic dengue cases are reported (World Health Organization, 1999b), while 50-90% of all dengue-infected individuals are asymptomatic (Balmaseda et al., 2006; Burke, Nisalak, Johnson, & Scott, 1988; Endy, Chunsuttiwat et al., 2002; Thein et al., 1997). Several previous studies have concluded that DHF and/or DSS occur predominantly during secondary infections with DENV2 viruses (Anantapreecha et al., 2005; Recker et al., 2009; Sangkawibha et al., 1984; Srikiatkachorn, 2009). Generally, risk factors for generating DHF and DSS consist of pre-existing immunity from a past dengue infection, being of school age, host gene, sequential infected serotypes, and viral genotype (Gubler, 1998a; Halstead, 2007; Kurane & Ennis, 1992; Siler, 1926).

Theoretically, exposure to an infected *Aedes* mosquito assigns an individual's risk for contracting dengue. Although there are several factors beyond direct self-protection, avoiding mosquitoes and reducing the breeding sources in and around households and public spaces can help abate the risk to some extent. The risk of a dengue epidemic can be mathematically elucidated by using the basic reproductive rate of the virus ( $R_0$ ), which corresponds to the number of subsequent

infections that would be produced in a group of susceptible individuals via the introduction from one infected person (Anderson & May, 1991). When  $R_0$  is lower than 1, transmission is hindered. Endemicity occurs when  $R_0$  equals 1, while an increase in cases occurs when  $R_0$  is higher than 1. The higher the basic reproductive rate, the more eruptive the transmission. When herd immunity is low, the increase in infections observed will be higher for diseases with a high  $R_0$ . Also, diseases with high  $R_0$  values have a lower herd immunity threshold for producing epidemics. A herd immunity threshold is the maximum level of immunity beyond which transmission is interrupted (Fine, 1993). This mathematic model is very useful for dengue transmission capacity assessment. Specifically, it considers the number of female mosquitoes per human host, the human-biting rate of the mosquito species, the proportion of bites which originate an infection, the average duration of infection in humans, the proportion of bites of viremic humans that produce infected mosquitoes, mosquito mortality rates, and latent incubation periods in both hosts (Anderson & May, 1991; Ferguson, Donnelly, & Anderson, 1999; Halstead, 2008).



\*Adapted from *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*. 2nd edition. WHO, Geneva, 1997

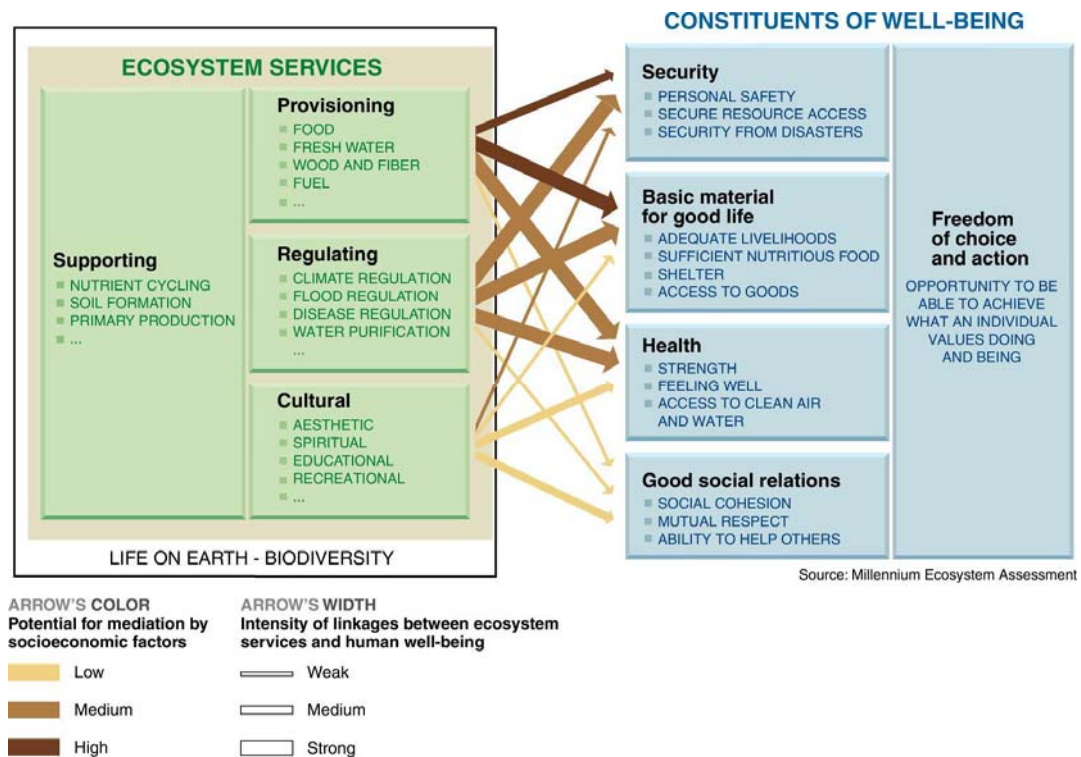
**Figure 4** An illness spectrum of dengue infection.

Image obtained from the website of CDC.org

## **2.2 Relevant Research on Ecological, Biological, and Socio-demographic Dimensions**

### **2.2.1 Ecological and climatic dimensions**

A global dengue emergence is a consequential outcome of three main environmental (eco-climatic changes), biological (dengue virus and *Aedes* mosquitoes), and socio-demographic factors. Among these underlying determinants, ecological and climatic drivers appear to play pivotal roles in the increase in *Aedes* mosquito vector as well as transmission of dengue. Ecology can be defined as the study of interactions among organisms and between organisms and their environment. There is much evidence, in particular, regarding ecologic and climatic determinants from which the expansion of *Aedes* mosquitoes and the emergence and reemergence of dengue result (Barrera, Amador, & Clark, 2006; Ellis & Wilcox, 2009; Thammapalo, Chongsuwiatwong, McNeil et al., 2005; Wilcox & Gubler, 2005). An understanding of the complexity of economic, ecological, and social systems is required for sustainable development. Hierarchies and adaptive cycles form the foundation of ecosystems and social-ecological systems across different scales. The ecological condition of regions, countries, or even smaller scales is a current item for assessment and action (Holling, 2001). The Millennium Ecosystem Assessment (MA) involved more than 1,360 devoted experts and demonstrated that human well-being and progress toward sustainable development absolutely depend on improving the management of Earth's ecosystems to ensure their conservation and suitable use. The MA not only clarified the consequences of ecosystem change for human well-being but also provided a state-of-the-art scientific appraisal of the status and trends in the world's ecosystems and the basic services they provide, as well as a suitable basis for governments, the private sector, and civil society to factor considerations of ecosystems and ecosystem services into their planning and actions (Millennium Ecosystem Management, 2009). Dengue fever is strongly linked to both provisioning services and regulating services, including those that influence the distribution of disease transmitting insects (Figure 5).

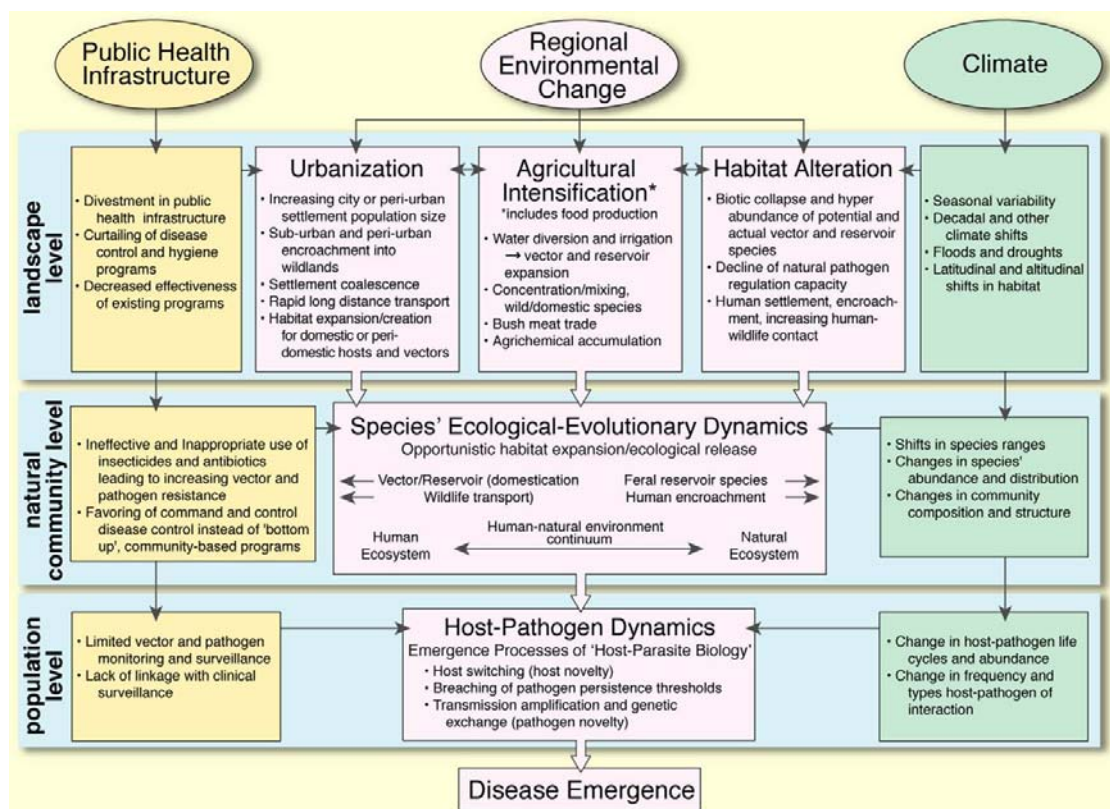


**Figure 5** Ecosystem services and their links to human well-being.

Image obtained from <http://www.millenniumassessment.org/en/Framework.aspx>

The hierarchical structure of nature and the positive correlation in the spatial and temporal scales of varying processes have become important issues of discourse in disease ecology (Collinge & Ray, 2006; Koopman & Longini, 1994; Marten, 2001; Turner, Gardner, & O'Neil, 2001). More precisely, there have been lines of evidence of association between degrees of urbanization along with habitat alteration and *Aedes* vector abundance, and therefore affecting the dengue transmission (Leishnam & Juliano, 2009; Schreiber et al., 2009; Strickman et al., 2000; Teixeira Mda et al., 2002). The broader, complex adaptive system view contributes to understanding the phenomenon of disease emergence, the ecological and evolutionary factors involved, and the role of parasitology in research and ecosystem management in light of the apparently growing problem of emerging infectious diseases in wildlife and humans (Horwitz & Wilcox, 2005). Wilcox and Gubler (2005) proposed a general model of global zoonotic disease emergence that links socio-demographic factors to land use and land cover changes whose associated ecological factors help explain disease emergence (Wilcox & Gubler, 2005).

The ecologically driven forces that affect disease emergence at different layers of ecosystems are based on three major drivers: public health infrastructure, regional environmental change, and climate (Figure 6). These basic drivers hierarchically affect disease emergence from the landscape and natural community level to the population level (Wilcox & Colwell, 2005).



**Figure 6** Multi-level framework on environmental factors affecting disease emergence. (Wilcox and Colwell, 2005, p.249)

Another example of a significant ecological driver is the role of ecotones that plays in infectious disease emergence. The boundaries between ecological systems known as ecotones have a long history in ecological science and zoonotic research (Despommier et al., 2006). A number of empirical investigations have shown an increase in dengue incidence resulting from changes in land use, and agricultural, demographic, and societal changes (Taylor, Latham, & Woolhouse, 2001; Woolhouse & Gowtage-Sequeria, 2005).

Climatic factors such as temperature, rainfall, and relative humidity have been proven to greatly impact the occurrence of dengue. Decreases in temperature have been clearly seen to shorten the duration of *Ae. aegypti* larval development in the laboratory, as well as in field observations (Angel & Joshi, 2008; Delatte, Gimonneau, Triboire, & Fontenille, 2009; Rohani, Wong, Zamre, Lee, & Zurainee, 2009; Tun-Lin et al., 2000; Watts et al., 1987). Additionally, some observations have shown a positive correlation between rising temperature, global warming, and increased dengue incidence (Bangs, Larasati, Corwin, & Wuryadi, 2006; Hsieh & Chen, 2009; Jury, 2008; Thammapalo, Chongsuwiatwong, McNeil et al., 2005), while other findings have revealed a significant correlation between high seasonal rainfall and dengue incidence (Chadee, Shivnauth, Rawlins, & Chen, 2007; Su, 2008; Wiwanitkit, 2006). Although various lines of climatic evidence provide insight into the significant drivers that contribute either directly or indirectly to dengue emergence, there are many underlying variables which remain obscure—for example, the reason for the great variability among these climatic factors that appears to occur in space and time. Insufficient observed data may be the reason for the uncertain findings of some research efforts. But recent studies have provided new evidence that multiyear climate variability, as well as intrinsic factors regulating transmission dynamics, may play a role in endemic interannual dengue dynamics (Cummings et al., 2004; Johansson et al., 2009).

A geographic information system (GIS)—which is a system for input, storage, manipulation, and output of geographic information—has been introduced as a capable tool for the prevention and control of dengue over the past decade. There is a superabundance of GIS software packages available, with various capacities for data processing, analysis, and display (Lozano-Fuentes et al., 2008). These powerful technologies have widely been used and proven useful for exploring and monitoring the prevention and control of dengue and its vector in space and time. In light of ecological and climatic drivers, these tools also have been proven powerful in exploring the influences of physiological-environmental factors on *Aedes* vector abundance and dengue incidence (Chansang, 2005; Honorio et al., 2009; Leisnham & Juliano, 2009; Nakhapakorn & Tripathi, 2005).

### 2.2.2 Biological dimensions

In keeping with the purposes of this study, the biological implications of dengue that are being reviewed throughout this section encompass two main dimensions: the dengue viruses and the *Aedes* mosquito vector. As long as a safe and competent tetravalent dengue vaccine or chemotherapy is unavailable, the prevention and control of the disease depend on vector control measures. Apart from ongoing dengue vaccine development, a large number of research attempts have investigated these fatal viruses mainly to catch up with their dynamics resulting from widespread outbreaks in nearly all countries of the tropical and subtropical regions.

Getting an insight into the variations found among dengue transmission patterns is fundamental to conducting surveillance and implementing disease prevention strategies. The clustering of dengue virus transmission has been proved by several studies (Kan et al., 2008; Kittayapong et al., 2008; Mammen et al., 2008; Thai et al., 2009; Thammapalo, Nagao et al., 2008). These findings confirm that a better understanding of spatial distribution is required for effective dengue interruption. An understanding of what biological factors play major roles in the dengue outbreak in a particular environment is also necessary for designing and implementing disease control strategies. Some research findings show that there appears to be different transmission patterns of serotypic-specific virus circulation in different areas (Endy, Chunsuttiwat et al., 2002; Graham et al., 1999; Nisalak et al., 2003; Peyerl-Hoffmann et al., 2004; Rahman, Tahmin, Mannan, & Sultana, 2007; Recker et al., 2009). Besides the various lines of research mentioned above, other authors have analyzed the correlation between serotype dynamics and disease incidence rates, as well as examined host determinants in relation to clinical manifestations and epidemiological patterns of dengue (Anantapreecha et al., 2005; Burke et al., 1988; Endy, Chunsuttiwat et al., 2002; Kurane & Ennis, 1992; Recker et al., 2009; Schreiber et al., 2009; Srikiatkachorn, 2009; Thein et al., 1997; Wichmann et al., 2004).

Entomological investigations and surveys are important for a mosquito-borne disease such dengue. Scientists and entomologists have put very strong efforts into discovering the mosquito determinants of dengue epidemics. The burning issues today do not center on *Ae. aegypti*'s overwhelming expansion of range but rather on the complex causal mechanisms underlying the origin and spread of fatal dengue

disease such DHF and DSS. In order to understand adequately the dynamics of dengue disease, we must perceive how and why vector populations change over time. Scientists have described various drivers that originate vector abundance and distribution. The available breeding containers in a particular area, variation of climatic and ecological factors in space and time, as well as the mosquito's own behavior appear to be the main drivers responsible for the intensity and distribution of the vector (Almeida et al., 2005; Chareonviriyaphap, Akratanakul, Nettanomsak, & Huntamai, 2003; Dutta et al., 1998; Moore et al., 1978; Tewari et al., 2004; Thavara et al., 2001). Consequently, accounting for variation in mosquito vector populations helps improve surveillance and prevention.

Since *Ae. aegypti*, the primary dengue vector, are capable of transmitting the virus with a notable efficiency, entomological thresholds are especially low. The risk assessment of dengue transmission based on immature mosquito indicators has proven difficult. Despite several lines of evidence showing strong relation between mosquito indices and dengue occurrence (Chang et al., 2009; Eisen & Lozano-Fuentes, 2009; Garcia-Rejon et al., 2008; Kittayapong & Strickman, 1993), there has been fierce debates about to what extent entomological indicators permit the transmission of dengue viruses (Halstead, 2008; Otero & Solari, 2009; Scott & Morrison, 2010).

### **2.2.3 Social and demographic dimensions**

Apart from a variety of underlying ecological and biological drivers, socio-demographic determinants as well as dengue control programs also greatly affect the transmission of dengue and the number and geographic expansion of its mosquito vector. An understanding of the complexity of socio-economic demographics and urbanization dynamics is a prerequisite for implementing successful dengue control strategies. Personal health beliefs and behavioral change models have long been used as a basis to design educational materials to encourage community participation in the prevention and control of dengue (Beckett et al., 2004; Lennon, 2005; Naeem-Ullah & Akram, 2009; Perez-Guerra, Zielinski-Gutierrez, Vargas-Torres, & Clark, 2009; Phuanukoonnon, Brough, & Bryan, 2006). However, several findings have demonstrated that while health education messages can raise awareness during a disease outbreak, they do not ensure sustained larval control practices (Caceres-

Manrique Fde, Vesga-Gomez, Perea-Florez, Ruitort, & Talbot, 2009; Itrat et al., 2008; Nalongsack, Yoshida, Morita, Sosouphanh, & Sakamoto, 2009; Van Benthem et al., 2002).

Barriers preventing successful control programs include insufficient control agents, inadequate knowledge of control methods, and incompatibility of control practices with people's beliefs (Caceres-Manrique Fde et al., 2009; Lennon, 2005; Rosenbaum et al., 1995). The promotion of community participation basically requires a discernment of the knowledge, attitudes, and practices of community members regarding the mosquito vector and dengue disease (Centers for Disease Control and Prevention, 1979; G. Kouri et al., 1998; G. P. Kouri et al., 1989; Perez et al., 2007; Teixeira & Medronho Rde, 2008; Thammapalo, Chongsuwiatwong, Geater, Lim, & Choomalee, 2005). Community participation for a source reduction/clean-up campaign has proven efficient when integrated vector control measures (e.g., screen covers, copepods, larvicidal agents and lethal ovitraps) are combined (Gubler & Clark, 1994; Kittayapong et al., 2008; Rosenbaum et al., 1995; Whiteford, 1997; Winch, Lloyd, Godas, & Kendall, 1991). Successful application of community-participation educational strategies to control the dengue vector have been described by several investigators (Haldar et al., 2008; Perez-Guerra et al., 2009; Regis et al., 2009; Sanchez et al., 2008; Schweigmann et al., 2009). Great emphasis has been placed on defining the function of water-holding containers within a family's daily activities. In addition, studies on community involvement have focused on perceptions, attitudes, and adopted practices viewed from several different settings and vantage points (Itrat et al., 2008; Naeem-Ullah & Akram, 2009; Nalongsack et al., 2009). Two relevant examples supporting such findings above encompassed the two cases of community and school-based health education for dengue control in rural Cambodia and a learning group and community working groups for dengue vector containment in municipal Cuba (Khun & Manderson, 2007; Sanchez et al., 2008).

Demographic risk factors for health problems are also essential to take into consideration prior to implementing any public health interventions (Aagaard-Hansen, Sorensen, & Chaignat, 2009). For example, children under 15 years have always been identified as a risk group for dengue and targeted for educational control programs (Khun & Manderson, 2007; Phuong et al., 2008; Wichmann et al., 2004).

The demographic and social changes that have occurred over the past 50 years have been strongly associated with the resurgence of dengue during this period (Gubler, 1998b; Guha-Sapir & Schimmer, 2005; Guzman & Kouri, 2003, 2008). Another main factor responsible for the global emergence of DF and DHF is an increase in air travel, which provides an ideal mechanism for conveying the dengue pathogen among population centers throughout the world. As a result, human movement is often considered a risk behavior factor underlying the transmission dynamics of dengue and other vector-borne diseases (Adams & Kapan, 2009; Cummings et al., 2009; Stoddard et al., 2009).

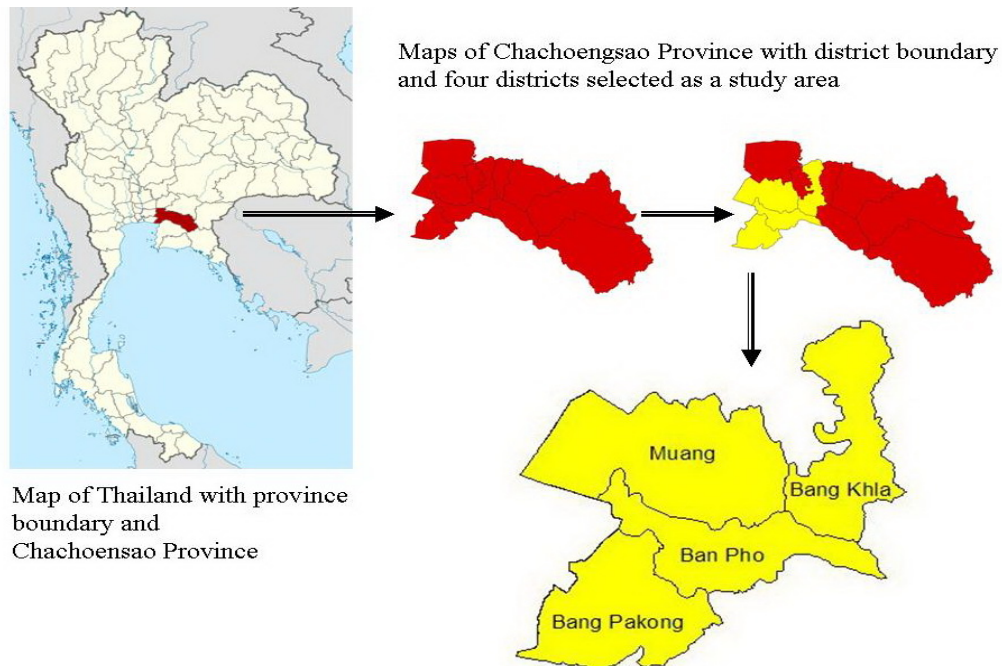
Although many successes of community-based dengue control projects have been achieved, their sustainability seems uncertain. Attention to the sustainability of public health intervention programs has been increasing in the recent years. Even so, consensus has not been reached regarding the conceptual and operational definitions needed for sustainability (Pluye, Potvin, Denis, & Pelletier, 2004; Shediach-Rizkallah & Bone, 1998). Recently, an excellent report has offered a framework for evaluating the sustainability of community-based dengue control projects (Hanh, Hill, Kay, & Quy, 2009). The framework applies 13 criteria, grouped into three categories: maintenance of health benefits from the original project, continued delivery of community activities, and human resource development. This framework provides a workable method for assessing sustainability, and is tractable to adaptation for specific interventions without compromising the framework as a whole.

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Study Site

Given the complexity of its geographic, socio-economic, and epidemiological conditions, Chachoengsao Province was representatively selected as a study site (Figure 7). It is situated approximately 75 kilometers east of Bangkok and its area covers 5,370.28 square kilometers. The four main types of land-use/land-types are wetland, riverine landscape, flatland, and mountainous area. Both urban and semi-urban communities are situated in low-lying, generally flat areas surrounded by rice fields and orchards. The climate in this region is characterized by a long rainy season (June-October), a winter dry season (November-January), and a hot dry season (February-May). In 2008, the average temperature was 27.96 °C, the average annual precipitation was 1,284.06 mm over 120.8 days, and the average humidity was 76 % (Thai Meteorological Department, 2009).



**Figure 7** Maps of study area: 4 districts including Muang, Bang Pakong, Ban Pho, and Bang Khla. Map of Thailand obtained from Wikimedia.org

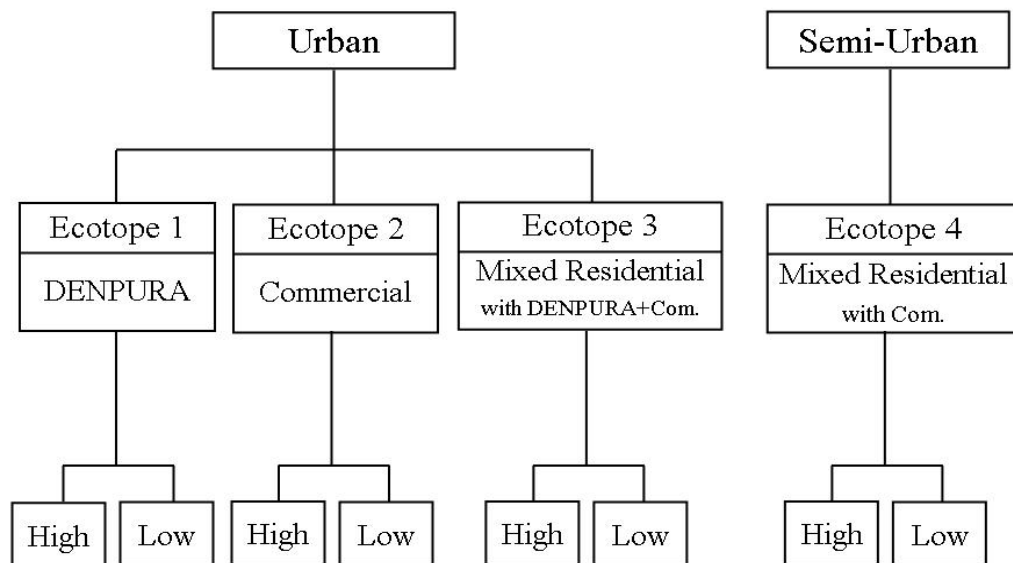
Administratively, Chachoengsao Province is divided into 11 districts: Muang, Bang Khla, Bang Nam Priao, Bang Pakong, Ban Pho, Phanom Sarakham, Ratchasan, Sanam Chai Khet, Plaeng Yao, Tha Takiap, and Khlong Khuean. In general, the urban region contains more dense households and has better infrastructure than the semi-urban parts. Like any other province in Thailand, the basic infrastructure includes connecting roads, electric service, and a tap water supply. The total population in 2008 was 651,785 (males 320,363 and females 331,422). Birth and death rates (per 100,000 population) were 1,038.22 and 192.70 respectively (Chachoengsao Provincial Public Health Office, 2009).

Dengue (DHF) incidence exhibits a strong seasonal pattern, with high transmission occurring during the rainy season. The yearly-reported dengue cases during the past decade show a fluctuating trend with major outbreaks in 1997 and 2001, which coincide with outbreaks in other provinces in Thailand (Bureau of Epidemiology. Department of Disease Control. Ministry of Public Health, 2009).

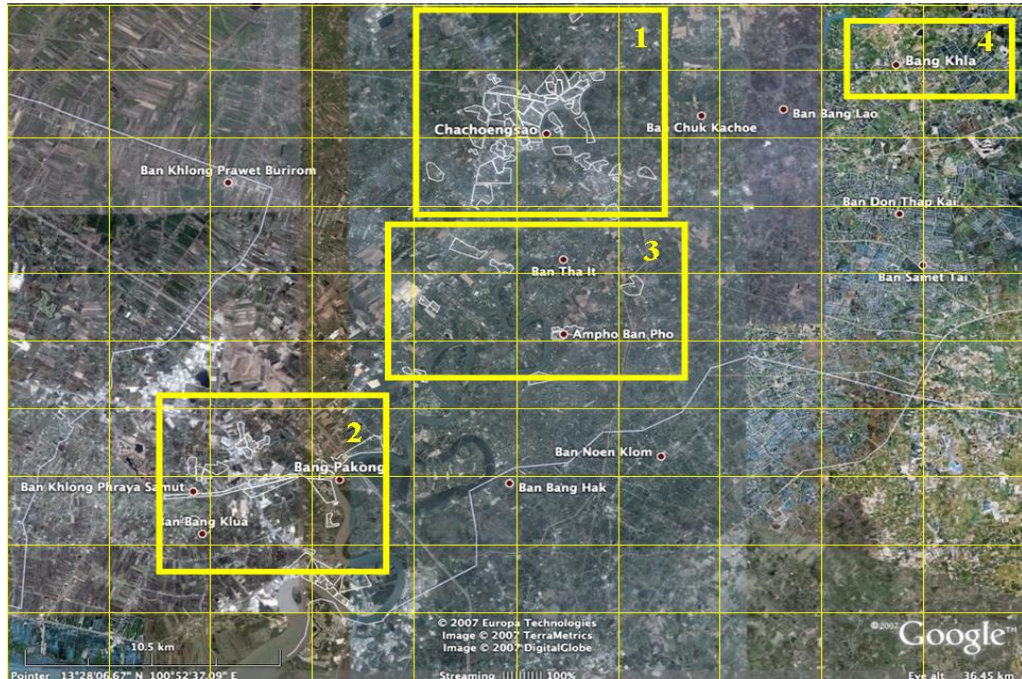
### **3.2 Study Clusters**

The selection criteria for the study clusters were based on the degree of urbanization and the intensity of dengue transmission, as shown in Figure 8. The degree of urbanization can be determined by applying the ecotope concept, which uses the smallest ecologically-distinct features in a landscape mapping and classification system. In this study, two main parameters were used to identify ecotope(s): the distribution of houses and the household in relationship to its environmental surroundings. Four ecotopes were chosen as study areas: densely populated urban residential areas (DENPURA), commercial areas, and mixed residential (commercial plus DENPURA and commercial alone). The intensity of dengue transmission was classified by using epidemiological data of dengue incidence obtained from the Ministry of Public Health and the Local Public Health Office in Chachoengsao Province. The levels of intensity were categorized into high and low degrees of dengue transmission.

Two-stage random sampling was applied for selecting targeted households. 120 clusters of approximately 100 houses each were assigned across 4 different districts of Chachoengsao Province (Figure 9). Then 12 out of the 120 clusters were randomly selected according to two criteria. The 6 clusters classified as urban were selected from the Municipality of Muang District, the Capital of Chachoengsao Province, while the other 6 clusters were chosen from semi-urban regions located in the Subdistrict Municipalities of 3 Districts: Bang Pakong, Ban Pho, and Bang Khla. Two representative clusters were separately selected from each of 3 different districts belonging to semi-urban areas. Eventually, the households targeted for the interdisciplinary surveys included a total of 1,200 households derived from 12 selected clusters multiplied by 100 households.



**Figure 8** Diagram showing selection criteria for the clusters: 1) degree of urbanization, and 2) intensity of dengue transmission.



**Figure 9** Google Earth Map showing cluster boundaries in 4 different districts of Chachoengsao Province: 1) Muang, 2) Bang Pakong, 3) Ban Pho, and 4) Bang Khla.

### 3.3 Sample Size

The sample size of the multi-stage sampling was calculated as per the formula set by Lwanga & Lemeshow (Lwanga & Lemeshow, 1991):

$$n = \frac{Z_{\alpha/2}^2 \times \pi \times (1 - \pi)}{d^2} \times \text{Design effect},$$

where  $n$  = sample size of multi-stage sampling

$$n_{\text{srs}} = \text{sample size from simple random sampling} = \frac{Z_{\alpha/2}^2 \times \pi \times (1 - \pi)}{d^2}$$

$\pi$  = population's proportion of IgG-IgM-positive school children (19.2%) in Chachoengsao Province (Kittayapong et al., 2008).

$d$  = allowance error (3%)

$Z_{\alpha/2}$  = a standard value from the standard normal distribution at a confidence level of 0.05 (1.96),

and Design effect = a ratio of the sample variance in a complex sampling and the sample variance in a simple random sampling. To be conservative, the design effect is assigned as 1.5.

Taken altogether, the sample size used in this study is mathematically expressed as:

$$n = \frac{(1.96)^2 \times 0.192 \times 0.808}{(0.03)^2} \times 1.5$$

$$n = 993.28 \approx 994$$

Adding 20% for missing data, the sample size is  $1,191.94 = 1,200$

Therefore, 1,200 subjects were collected from urban and semi-urban areas of Chachoengsao Province. Thanks to the multidisciplinary approach of the study, 1,200 household samples were used for entomological and ecological surveys. At the same time, a socio-epidemiological survey was conducted by interviewing 1,200 respondents, each of whom was randomly selected from the eligible household members of individual houses. The eligible respondents had to meet the following criteria.

#### **Inclusion criteria**

1. Household member living in the house at least 6 months per year.
2. Male or female aged  $\geq 18$  years.
3. Be able to communicate and willing to participate in interview.
4. No hearing loss.

#### **Discontinuation criteria**

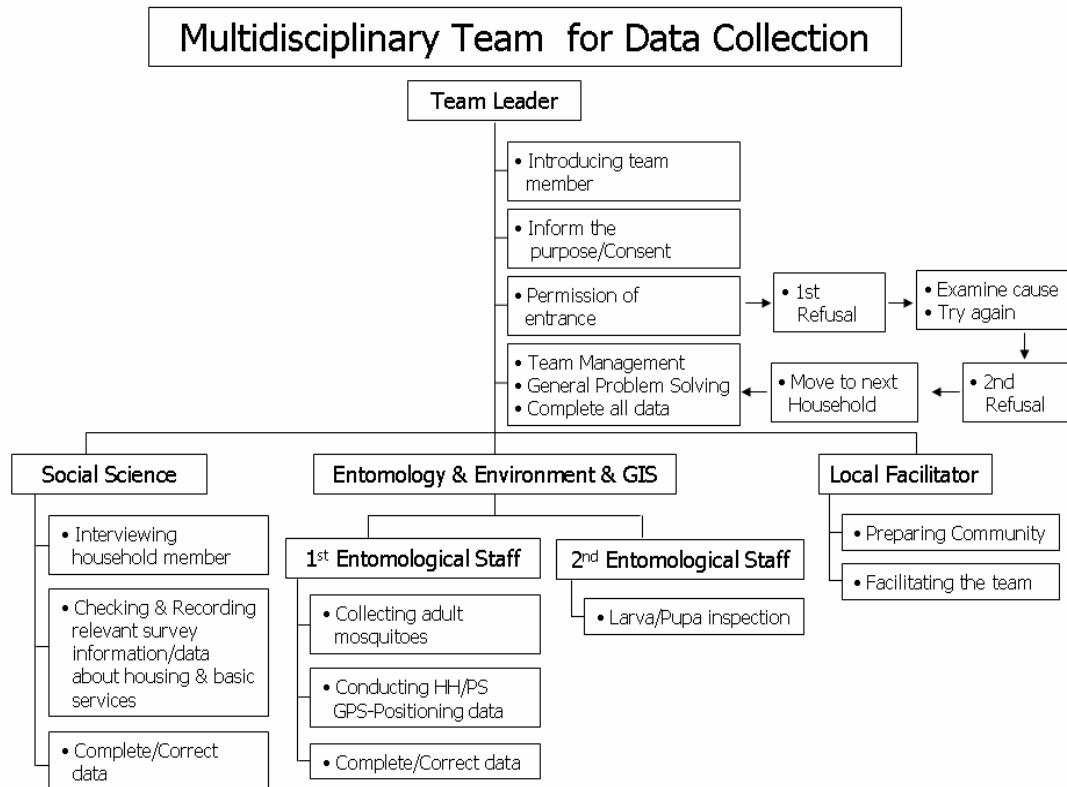
1. Involuntarily serving as an interviewee or unintentionally allowing for entomological and environmental survey without receiving permission.
2. Participants are free to discontinue the participation at any time for any reason without informing the researcher.

### **3.4 Multidisciplinary Team**

The sociological, entomological, and ecological field surveys were performed by a multidisciplinary team. The team was composed of four members: one conducting a socio-epidemiological survey, one collecting GPS-positioning data and adult mosquitoes inside houses, one conducting entomological and environmental surveys, and one coordinating and communicating with the community (Figure 10).

All team members were trained extensively so that they understood the study objectives and survey concepts, and thus could perform their tasks successfully. Shortly after training, they conducted exercises towards the pilot survey in other

different urban areas in the province to ascertain whether their skill sets were achieved to meet desired performance targets. The house-to-house surveys spanned out from the epicenter within the cluster. Throughout the field surveys, the principal investigator took responsibility over data collection and quality control, and supervised the specific tasks of others on the team.



**Figure 10** Multidisciplinary team involved in the data collection.

### 3.5 Socio-Epidemiological Survey

The socio-epidemiological surveys at the household level were conducted in order to evaluate the history of dengue outbreaks, the housing structure together with its basic services, and the socio-demographic determinants. A set of socio-demographic data encompassed population characteristics; knowledge about dengue haemorrhagic fever and its vector; and beliefs and practices regarding the roles and responsibilities of households, local authorities, and health workers for preventing and controlling dengue, as well as the effectiveness of existing control measures and “should-do” desired activities.

All these data were collected using a household questionnaire (Appendix A) that consisted of 8 main parts: 1) survey information, 2) household georeference, 3) demographic characteristics of head of household and respondent, 4) household composition and past experience of dengue, 5) housing and basic services, 6) knowledge about dengue and its vector, 7) attitude relevant to dengue and vector control, and 8) vector control practices. The questionnaire was designed with respect to study objectives, content analysis, and validity, as well reliability. All various study concepts were validated by three experts in the fields of ecology, medical biology and entomology, and social science. To test its reliability, the Alpha model with an acceptable level, Coefficient alpha = 0.7, was used for dichotomous data.

Using this validated questionnaire, a total of 1,200 targeted households in 12 clusters were examined with the assistance of well-trained professional nurses and /or public health officers, as part of the multidisciplinary teams. The one eligible respondent of each household was a family member 18 years of age or older. All volunteers randomly recruited into the study gave their informed consent after they were completely informed about the study's purposes, as well as the advantages and disadvantage of participation.

### **3.6 Stakeholder Analysis**

As part of the social science research, stakeholder analysis in relation to functions and interests was carried out for triangulating with household and dengue vector survey data and ecotope assessments in terms of contextualized eco-bio-social system analysis. In the study area, stakeholders of the respective agency or group involved in the dengue and vector control problem were recruited for interviews by using a stakeholder analysis matrix form (Appendix B). The stakeholder analysis matrix form was designed to cover two main aspects: 1) information received from the stakeholder, and 2) researcher impression. The first aspect included the main mission or activities of stakeholder; the main activities in relation to dengue and their proportion to the entire activities; the budget and source of dengue control activities during the previous year; identifying other collaborating stakeholders; and the particular successes or problems. The other aspect covered not only researcher impression but also further comments.

All interviews were tape-recorded and detailed notes were simultaneously taken. Tape-recorded interviews were transcribed according to standard rules (MacLean, Meyer, & Estable, 2004) and translated from Thai into English. Stakeholder's information from a variety of published and unpublished sources, such as journals, books, manuals, annual reports, and organizational charts were combined in case the stakeholder information was not completed by an interview. Once the information pertaining to all of the stakeholders involved in the dengue and vector control problem had been completely obtained, these stakeholders were identified, profiled, and then linked to the dengue problem assessment in the eco-social context by applying SAS2 Social Analysis Techniques (Social Analysis Systems<sup>2</sup>, 2010), as specifically described below.

Stakeholder identification and description were carried out according to the checklist method. After different stakeholder groups had been identified and described, their particular interests and positions were profiled using the Social Analysis CLIP (Collaboration and Conflict, Legitimacy, Interests, Power) technique. These profiles were based on four factors: 1) existing relations of collaboration and conflict, 2) legitimacy, 3) interests, and 4) power. The advantage of this technique is that it can describe the characteristics and relationships of key stakeholder in a concrete situation and scrutinize ways to address social problems in relation to dengue. The last step of the stakeholder analysis was problem assessment. In this study, the problem tree method was used to describe, identify, and situate the dengue problem in the context of comprehensive eco-bio-social factors as underlying determinants.

### **3.7 Cluster Information Survey**

Besides the socio-epidemiological survey of the 1,200 households located among the 12 studied clusters, each cluster was generally examined by using a survey form to obtain cluster background information (Appendix C). Relevant aspects included in the cluster survey form were: general information about the cluster, density of population and green areas, basic infrastructure, public spaces, recent vector control activities, community participation, and ecological characteristics of adjacent areas of the surveyed cluster. The purpose of the cluster survey was to gain various sets of baseline data, which were used to identify influencing determinants of dengue

transmission and its *Aedes* vector in the area by combining it with other lines of obtained knowledge.

### **3.8 GIS Mapping of Study Households**

The locations of all sampled houses were measured with a Global Positioning System (GPS) unit (eTrex Legend®, Garmin International, Inc., Olathe, KS) which has a distance error of  $\pm 3$  meters. The data recorded in the field were transferred to a laptop that had the MapSource software application (MapSource software version 6.15.7, Garmin International, Inc., Olathe, KS) for Geographic Information System (GIS) manipulation of field data. Finally, the field data were transferred in the laboratory work station to another personal computer that had GoogleEarth map and the digital base map from the Royal Thai Survey Department at a scale of 1:50,000 and Arcview software (ESRI, Redlands, CA) for manipulating them with relevant data. The GoogleEarth maps not only showed household locations in the study areas but also demonstrated spatial household distribution in terms of herd immunity of community members inhabiting different clusters.

### **3.9 Entomological Surveys**

Entomological surveys of the entire 1,200 households situated in each cluster were carried out during the wet and dry seasons to evaluate the container types, their social use, and the productivity in terms of larva/pupa density, as well as to assess how dengue mosquito abundance was associated with relevant factors. The entomological survey form for households (Appendix D) covered two main areas: mosquito breeding habitat and larva/pupa density.

Larva and pupa collection was done according to the method developed in other similar studies in Thailand and other countries (Barrera, 2009; Seng, Seta, Nealon, & Socheat, 2009; Strickman & Kittayapong, 2002, 2003; Tun-Lin, Kay, & Burkot, 1994; World Health Organization, 1995). Briefly, water storage containers (jars and cement bath basins) and drinking water containers were examined with a flashlight, and the presence of the immatures recorded. All visible pupae were hand-collected or by dipping. The *Aedes* immatures in other containers (tires, flower pots, discarded containers, ant traps, etc.) were sampled by dipping with a fine-meshed

fishnet (diameter of 25 centimeters) or, for the smallest containers, by pouring out the water into a bottle. To collect the larvae in containers, the dipping started from the top of the container and continued to the bottom in a swirling motion covering all edges of the container. The number of pupae in each container was recorded.

Entomological indicators used in this study included House Index (HI), Container Index (CI), Breteau Index (BI), and pupae per person (Focks, 2004; Focks, Brenner, Hayes, & Daniels, 2000; Focks & Chadee, 1997).

House Index: Number of houses positive for *Ae. aegypti* larvae and/or pupae per 100 houses surveyed.

Container Index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 potential habitats examined.

Breteau Index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 houses surveyed.

Pupae per Person: The ratio of *Ae. aegypti* pupae to human density.

### **3.10 Investigation of Dengue Herd Immunity of Human Population by Using Mosquito Blood Meal**

#### **3.10.1 Adult mosquito collection and identification**

Regardless of the potency of the vector of dengue virus, wild adult mosquitoes were collected indoors and around 100 individually surveyed houses among the 12 studied clusters during the wet and dry seasons. By using an electric handheld aspirator, entomologically well-trained field staff in each team performed adult mosquito collection. The collected mosquitoes were transferred into a sealed plastic bag in an icebox. Immediately after cold temperature inactivation, treated mosquito(es) were afterwards placed into labeled eppendorf tube(s). At the end of field work each day, all mosquito specimens were finally transferred under the same conditions to the laboratory at the Center of Excellence for Vectors and Vector-Borne Diseases (CVVD), Faculty of Science, Mahidol University at Salaya, where they were stored at refrigerated temperature. Within 24 hours after the arrival of each sample lot, all adult mosquitoes were microscopically examined by well-trained laboratory technicians for species and sex identification, and blood meal. Subsequently, all these

completely-identified samples were individually kept in labeled eppendorf tubes and finally placed into a box for storage at  $-80^{\circ}\text{C}$  until later use.

### 3.10.2 Human blood meal characterization

An enzyme-linked immunosorbent assay, which is specific for human IgG (antibody-captured), was used to examine the presence of human IgG in blood-engorged *Culex* mosquito samples according to the methods previously described (Barbazan, Palabodeewat, Nitatpattana, & Gonzalez, 2009; Innis et al., 1989; Kuno, Gomez, & Gubler, 1987). These phlebotomical *Culex* mosquitoes were further used to identify anti-Dengue virus human IgG and IgM. Briefly, the 96-well polystyrene microtiter plate was incubated overnight at  $4^{\circ}\text{C}$  with  $50\ \mu\text{l}$  of purified goat anti-human IgG antibody (KPL, USA) as the primary antibody. The plate was blocked with 0.5% casien in phosphate-buffered saline (PBS) solution (pH 7.4), followed by washing three times with an excess volume of PBS pH 7.4 in the presence of 0.05% Tween-20 (PBT). Subsequently, an individual blood-engorged mosquito was ground in  $200\ \mu\text{l}$  PBS pH 7.4 to make a dilution of 1:50. Then, using PBT, it was prepared to make a working dilution of 1:200.  $50\ \mu\text{l}$  of diluted mosquito lysate (1:200) was added into each well of the pre-coated plate. The mosquito lysate with the same working dilution from the laboratory strain of human blood-fed *Culex* was treated as the positive control, while purified goat anti-dog IgG antibody was used as the negative control. For internal control, a phosphate-buffered saline (PBS) solution with 0.5% casien and 0.05% Tween-20 was also used. All the samples were done in duplicate. After incubation at  $37^{\circ}\text{C}$  for one hour, the reactions were washed 3 times with PBT.  $50\ \mu\text{l}$  of goat peroxidase-conjugated anti-human IgG antibody (1:20,000) (KPL, USA) diluted in PBT was added into the wells. The reactions were incubated at  $37^{\circ}\text{C}$  for one hour, followed by washing three times with PBT. For spectrophotometric analysis,  $100\ \mu\text{l}$  of o-phenyldiamine dihydrochloride (OPD) solution as substrate, prepared in citrate phosphate-buffered solution in the presence of 3% hydrogen peroxide, was added into the wells. Immediately after incubation at room temperature for 30 min in a dark room,  $50\ \mu\text{l}$  of 4 M  $\text{H}_2\text{SO}_4$  as a stop solution was added into all wells, and subsequently the reactions were read at a wave length of 490 nm. The positive reaction arbitrarily determined for human IgG antibody must show a significant optical density

(OD) greater than mean cutoff OD plus two standard deviations of the negative controls. The average OD of negative controls was derived from 42 unfed female *Culex* mosquitoes.

### **3.10.3 Examination of anti-dengue immunoglobulin (Ig) G and IgM antibodies**

Human blood-fed mosquito samples were used to examine the presence of anti-dengue IgG and IgM antibodies, utilizing the commercially available SD BIOLINE Dengue IgG/IgM rapid test (Standard Diagnostics, Inc., Korea), which relies on anti-dengue virus antibody capture for all 4 serotypes by a mixture of colloidal gold-conjugated recombinant dengue envelope proteins. Briefly, 5 µl of each of the positive samples were added into the sample well, followed by adding diluent into the assay diluent well of the card test. In an anti-dengue IgG/IgM positive sample, the anti-dengue IgG/IgM-captured gold conjugates migrate up to the membrane across the pre-coated mouse monoclonal lines specific for human IgG (G line) and IgM (M line) antibodies, forming within 20 minutes one or two pink lines for the interpretation of the clear-cut test results.

For an IgM-positive sample, the accumulation of the gold conjugates will appear strong or pale pink in color at the M line in the absence of a pink line forming at the G line. This test result will be an indication that human blood-fed mosquito(es) have been exposed to recent or active dengue infection in the human population.

For an IgG-positive sample, the accumulation of the gold conjugates will appear strong or pale in pink color at the G line in the absence of a pink line forming at the M line. This test result will be an indication that human blood-fed mosquito(es) have been exposed to past dengue infection in the human population.

For an IgM and IgG-positive sample, the pink lines will form on both M and G lines, indicating that human blood-fed mosquito(es) may have been exposed to either late active or early past dengue infection and early secondary/tertiary/quaternary infection in the human population.

For validation of rapid diagnosis in all cases, the control line (C) will always appear as a pink line, as a result of the free gold conjugates being captured by

rabbit anti-Dengue IgG. If the invalid C line occurs, the same 5  $\mu$ l each of positive samples will be repeated by using a new card test.

### 3.11 Data Analysis

Throughout this study, the SPSS statistical program, version 17.0 (SPSS, Inc., Chicago, IL), was used for mainly quantitative data analyses. The quantitative data obtained from socio-epidemiological, entomological, and environmental surveys, as well as the examination of anti-dengue IgG and IgM antibodies, were cleaned up and later entered into the SPSS software package for performing the data analyses. The locations of surveyed houses measured by the GPS device were loaded into the Arcview GIS program for creating the GoogleEarth maps. The qualitative data gained from stakeholder interviews and relevant documents were prepared and analyzed by using the Atlas.ti-The Knowledge Workbench, version 6.0 (1993-2009 ATLAS.ti Scientific Software Development GmbH, Berlin).

The univariate analysis was used to examine predictors of various ecotopes for mosquito vector abundance and the emergence of dengue in human hosts. These independent binary variables and their two dependent parameters were calculated by applying Odds Ratios (OR) and their 95% confidence interval (95%CI).

For entomological data, Chi-square tests of homogeneity were performed to evaluate the distribution of frequencies relating to container types and their social use, productivity, and seasonality.

The Chi-square test was used to compare DENV seropositivity (%) of *Culex* mosquito blood meals among 4 ecotopes in relation to nominating factors like urbanization (urban and semi-urban) and dengue transmission level (high and low).

A univariate analysis of Odds ratio (OR) and 95% CI was used to analyze associations or estimate individual risk factors including ecological and sociodemographic determinants for dengue case history (dengue transmission) and pupa demographic index. A significant OR ( $P < 0.05$ ) or OR greater than 1.5 times was considered risk.

Multiple logistic regression analysis driven by a holistic interdisciplinary eco-bio-social approach was further used to model multifactorial risk factors attributed to dengue transmission.

In addition, the human herd immunity against dengue virus serotypes co-circulating in *Culex* mosquito populations within each of the ecotopes was determined in terms of active and past dengue infection, using univariate analysis.

### **3.12 Ethical Review and Approval**

As part of a WHO/TDR/IDRC-funded multi-country study led by my major advisor, Associate Professor Dr. Pattamaporn Kittayapong, who also served as the principal investigator, my Ph.D. research was determined to be research with exemption in the Institutional Review Board (IRB) review conducted by the Mahidol University Institutional Review Board [COE. No. MU-IRB 2010/019.2505] (Appendix E). The ethical clearance of the multi-country study had earlier been reviewed and approved by the Committee on Human Rights Related to Human Experimentation, Mahidol University, Bangkok [COA. No. MU-IRB 2009/162.2307]. (Appendix F).

The study was undertaken in compliance with the “International Guidelines for Human Research Protection” detailed in the Declaration of Helsinki, The Belmont Report, the CIOMS Guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). Confidentiality of each respondent was ensured, and any possible ethical concerns were discussed by the multidisciplinary team members, me, and my major advisor prior to starting the survey. Written consent to participate in the surveys was obtained from all individual participants.

## CHAPTER IV

### RESULTS

This Ph.D. research was part of a WHO/TDR/IDRC-funded multi-country study led by my major advisor, Associate Professor Dr. Pattamaporn Kittayapong, who also served as the principal investigator. The data were mainly collected by multidisciplinary teams using instruments developed from a series of community practice workshops delivered through a multi-country network in Asia.

Applying various sets of instruments to this research, all field data surveys and mosquito sample collections were carried out among 12 study clusters in 4 districts of Chachoengsao Province from mid-year 2007 until mid-year 2008. The laboratory blood meal analysis was performed in 2009. In addition to these research activities, stakeholders involved in dengue and/or dengue vector control were interviewed in 2010 by using a stakeholder matrix form. All sets of acquired data were processed and analyzed during 2008-2010.

Socio-epidemiological surveys at the household level, as well as GIS mapping, were undertaken for 1,200 houses belonging to 12 clusters during August-October 2008. At the same time, entomological surveys in conjunction with adult mosquito collections were conducted during this period of the rainy season. Cluster background information was also collected in 12 clusters of the study area in 2008. During the dry season (March to April) of the following year, entomological surveys and adult mosquito collections were repeatedly carried out for the entire houses previously surveyed. Using *Culex* mosquito blood meals collected during the wet and dry seasons, a laboratory investigation of dengue herd immunity of human populations was conducted in 2009. Additionally, a stakeholder analysis was carried out during May-July 2010, so that results could be described, identified, and situated in their local eco-social context. Lastly, by applying appropriate statistical programs and relevant tools, a series of data analyses were carried from the end of the 2008 field surveys, until the end of July 2010.

This chapter details various sets of research findings derived from the interdisciplinary eco-bio-social instruments. The ultimate goal of this study was to assess dengue transmission dynamics in urban and semi-urban settings in Chachoengsao Province. All research findings, which encompass 3 main eco-bio-social dimensions, have been organized into 5 principal sections. The first section describes the characteristics of 12 study clusters in terms of cluster backgrounds, such as infrastructural, socio-economic, spatial characteristics. The second section portrays a set of ecological determinants as risk factors for dengue case history and an entomological index. Results of entomological and blood meal analyses incorporated into biological implications to dengue transmission dynamics are demonstrated in the third section. In the fourth part, findings of socio-demographic parameters in relation to the degree of dengue transmission, as well as stakeholder analysis, are discussed. The last section addresses the results of our socio-ecological analysis in relation to dengue occurrence and the multivariate analyses of the socio-demographic factors that underlie dengue transmission in a significant ecotope of residential areas mixed with commercial areas and densely populated urban residential areas (DENPURA).

#### **4.1 Cluster Characteristics**

Twelve clusters covering 4 distinct ecotopes with high and low degrees of dengue transmission in urban and semi-urban settings randomly-stratified and selected from 4 out of 11 districts in Chachoengsao Province (Table 1) were used to assess dengue transmission dynamics via a holistic eco-bio-social approach. All studied neighborhoods were sorted into various ecotopes, which differ by the socio-economic status (SES) of local inhabitants, infrastructural services, land use, and land type (Figure 11-22). The ecotope of a densely populated urban residential area or DENPURA is characterized by the low socio-economic status of the population and the relatively low locus on matters related to community development, including social and economic opportunities, access to health care resources, as well as infrastructural water supply and waste management, and a lack of vegetation and/or forested areas. The commercial ecotope (C) denotes a high SES of occupants and good basic infrastructures, along with a great deal of commercial and business buildings. Unlike the DENPURA and commercial ecotopes, the ecotope of residential (R) mixed

with commercial and DENPURA (RCDENPURA) exhibits a main residential zone prominently scattered with commercial and DENPURA, while the residential mixed only with commercial (RC)—signifying an overlap of urban and rural areas—is an area mainly for dwelling but which is also filled in partly with commercial sections.

**Table 1** General characteristics of study clusters in Chachoengsao Province.

Characteristics	Number of clusters		Total N=12
	Degree of dengue		
	High	Low	
<b>Ecotope (N=12)</b>			
DENPURA	1	1	2
Commercial area	1	1	2
Residential mixed with commercial area and DENPURA	1	1	2
Residential mixed commercial area	3	3	6
<b>Urbanization (N=12)</b>			
Urban area	3	3	6
Semi-urban area	3	3	6

Using a well-developed survey form, a cluster background survey was carried out in each selected study cluster and adjacent areas to obtain various sets of basic information within the clusters. All houses, as well as public spaces located in each cluster, were included in the cluster analysis. The overall socio-economic, infrastructural, and spatial characteristics of the study clusters are individually described as follows.

**4.1.1 Cluster 1: Soi Li-Ke**

Soi Li-Ke is an urban residential mixed with commercial area and DENPURA, with a high degree of dengue transmission. The overall socio-economic status of the local inhabitants is considered a blend of upper middle and lower economic strata. Most of the houses are 2-storey and in fair condition structurally.

The mean distance of nearest houses is approximately 3.20 meters. There are some green areas, a few small pools larger than 5 square meters and one beverage company located in this cluster. The basic infrastructure includes adequate water supply, electricity, and daily garbage collection by the municipality of Chachoengsao. However, some small garbage dumps were observed alongside alleys in the cluster. The local inhabitants access the area via a main concrete road and alleys. This cluster is surrounded by residential and commercial communes and some of the community members often leave home during the daytime to work outside the cluster.

#### **4.1.2 Cluster 2: Ta Kham**

Ta Kham is an urban residential mixed with commercial area and DENPURA, with a low dengue transmission degree. The socio-economic status is also a mix between upper middle and lower economic strata. A majority of houses are 2-storey and in fair condition structurally. The mean distance of nearest houses is approximately 3.54 meters. The cluster location is partly adjoining the Bangpakong River. There are some green areas but no pools larger than 5 square meters or public spaces are in the cluster. The basic infrastructure includes adequate water supply, electricity, and daily garbage collection by the Chachoengsao municipality. However, some small garbage dumps were observed alongside alleys in the cluster. Besides three private companies, there were no public spaces observed during the study. The dwellers access the area via a main concrete street and alleys, and some of them leave home to work outside the cluster.

#### **4.1.3 Cluster 3: Wannaying**

Wannaying is an urban neighborhood located within a densely populated residential area of Chachoengsao Province. The degree of dengue situation has recently been high for this cluster. The socio-economic status is the lowest economic stratum among the studied clusters. Most of the houses contain 2-storey and poor housing structures with bad sanitation. Housing distribution is crowded and the mean distance of nearest houses is about 0.41 meters. The cluster location is surrounded primarily by commercial zones, markets, and one commercial college. There are no spaces for green areas, vegetation, and any type of public spaces inside the area.

Nevertheless, the basic infrastructures such as electricity, water supply, and garbage collection are accessible by local residents. There are three main concrete streets that surround the cluster area and small concrete alleys connect the communities. Most of dwellers move daily in and out the community to earn their living at various workplaces and, consequently, leave children and aged people at home during the daytime.

#### **4.1.4 Cluster 4: Sapandam**

Sapandam is considered a densely populated urban residential area cluster with a low degree of dengue transmission. Recently, this original community was separated into large and small regions by the housing development. The socio-economic status is as low as the Wannaying community. A majority of houses in the selected area are elevated above scattered puddles and in poor condition structurally. The water in the puddles becomes less and more polluted during the dry season. The mean distance of nearest houses is approximately 0.85 meters. This cluster area is connected to the Ta Khai Canal, meadow, residential, and commercial areas. There are no spaces for green areas, vegetation, and any type of public spaces inside the area. Local inhabitants only have full access to electricity; most lack piped water and receive irregular garbage collection by the municipality. Some residents who have no access to the public water supply use water from their own rain tanks or pump water from the Ta Khai Canal. Because of an irregular garbage collection, some inhabitants dispose of garbage by burning it or throwing it in mounds around the houses. Access to the large main area is limited to a single main route, and only one narrow elevated concrete alley has been available for local residents to walk within this region. Due to an unavailability of work inside the region, nearly all working groups in this community leave home daily for their workplaces and, consequently, leave their children and elderly relatives at home during the daytime.

#### **4.1.5 Cluster 5: Mahajakapad**

Mahajakapad is one of the commercial zones situated in the urban area of Chachoengsao Province. In this area, the overall dengue transmission degree is ranked high. The socio-economic status of this population is highest of any community in the

study area. All of the households in this community are located along both sides of Mahajakapad Street. Almost all of houses, which are mainly used for commercial purposes, are 2-to 3-storey buildings and in very good condition. Furthermore, the majority of houses contain no gardens or outdoor spaces. The mean distance of nearest houses is approximately 0.10 meters. This community is situated in the heart of Chachoengsao City and has become a business/commercial zone. Local residents and visitors access this area for living, shopping, and doing business via the Mahajakapad main street and its connecting alleys. Except around a few government offices and one technical college, there are no green areas, vegetation, and other public spaces within the area. Residents have full access to electricity, a piped water supply, and waste management. Because of the availability of work inside the cluster, nearly all local residents work at or near their home, while other people outside the community move in and out to work, do business, and shop.

#### **4.1.6 Cluster 6: Tepkunakorn**

Tepkunakorn is an urban commercial zone with a low degree of dengue transmission. The population's socio-economic status is as high as Cluster 5, the Mahajakapad community. All households in this community are located along both sides of Tepkunakorn Street. The majority of houses, which are mainly used for commercial purposes, are 2-to 3-storey buildings, in very good condition, and have no gardens or yards. The mean distance of nearest houses is approximately 0.04 meters. This community has recently become a business or commercial zone. Besides local inhabitants, visitors come to this area to work, shop, and do business via the principal street (Tepkunakorn) and its connecting alleys. There are no green areas, vegetation, and other public spaces within the area. Electricity, piped water, and waste management are accessible infrastructures for dwellers in the community. Thanks to an availability of work inside the area, nearly all local residents work at or near their homes, while many people from different neighborhoods moved about to work, do business, and shop.

#### **4.1.7 Cluster 7: Burapa City**

Burapa City is a suburban neighborhood located within a residential mixed with commercial area. This area is situated about 0.50-kilometer away from the main street, and it is adjacent to many industrial neighborhoods. The epidemiologic situation of dengue in the area is considered to be high. The comprehensive socio-economic status of local inhabitants falls into the upper middle economic stratum. All selected households of this cluster are located among the more than 1,000 houses of the housing development. All houses are in the same pattern, a 2-storey building with a small yard and a surrounding fence wall. The mean distance of nearest houses is about 0.11 meters. This community has been recently established as a residential zone. Thus, nearly all dwellers are factory employees and workers with jobs outside the community. These people migrate from various places both inside and outside Chachoengsao Province. There is one green area for leisure, one nursery, and one business school inside the area. Electricity, piped water, and waste management are basic infrastructures for dwellers in the community. Because most inhabitants work outside the community, they leave children and aged persons at home, particularly during the daytime.

#### **4.1.8 Cluster 8: Ta Sa-arn**

Ta Sa-arn is a suburban residential mixed with commercial area. This area is situated around the Chachoengsao-Bangpakong highway and adjacent to the Bangpakong River and many industrial neighborhoods. The degree of dengue situation in the area is ranked low. The comprehensive socio-economic status of the local inhabitants is the upper middle economic stratum. There are various types of houses in this cluster; however, the 2-floor house is predominant and conditions are mostly good. The mean distance of nearest houses is about 1.31 meters. Some dwellers are not local people, and have migrated from various places both inside and outside Chachoengsao Province. There are some green areas and banks, private companies, one gasoline station, and one post office inside the area. This cluster is provided with a public water supply, electricity, and a daily garbage collection. The local inhabitants dispose of their garbage in containers designated for this purpose. These services are regular without interruptions.

#### **4.1.9 Cluster 9: Tepparachbureerom**

Tepparachbureerom is a suburban residential mixed with commercial area, with a high degree of dengue transmission. This community is situated along both sides of the Prawatebureerom Canal and surrounded by rural areas. Housing distribution in the cluster is mostly crowded at the center, while some homes are scattered around among meadows and shrimp farms. The comprehensive socio-economic status of local inhabitants varies from the upper middle to lower economic strata. There are various types of houses in this cluster; however, one storey houses prevail and their structures are mostly in fair condition. The mean distance of nearest houses is about 14.52 meters. There are various types of occupations among dwellers, but predominantly it is farm owners and their workers, and shop owners and their employees. There is one temple and one school inside the area. The households are provided with electricity, a public water supply, and daily garbage collection services. However, some residents still use water from their own rain tanks or pump water from either a well or the Prawatebureerom Canal.

#### **4.1.10 Cluster 10: Ban Sanamjan**

Ban Sanamjan has long been a settled suburban residential mixed with commercial area, with a low degree of dengue transmission. This ancient neighborhood is located alongside the Bangpakong River and the Nalang Canal. Many of the houses are attached, while some are scattered around the center of the community. The population's socio-economic status is between the upper middle and lower economic strata. There are various types of houses in this cluster, but two-storey houses are predominant and their structures are mostly in good condition. The mean distance of nearest houses is about 5.56 meters. Some residents are shop owners, while the rest are mostly laborers and employees, most of whom work outside the community. There is one Sub-district Municipality situated inside the area. All households are provided with electricity, a public water supply, and garbage collection services. Still, some residents disposed of garbage by burning or throwing it in mounds around the house.

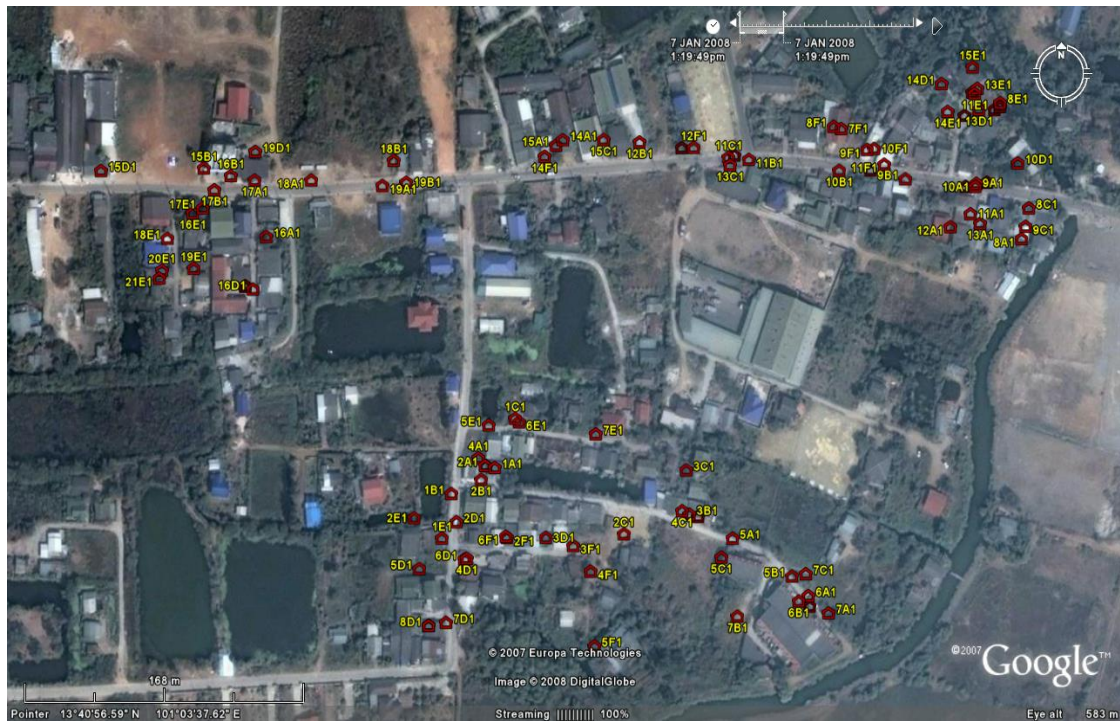
#### **4.1.11 Cluster 11: Lang Wat Jaeng**

Lang Wat Jaeng is a sub-urban residential mixed with commercial region, with a high degree of dengue transmission. This community is situated alongside the Wat Jaeng Canal and has 4 main paved streets with their connecting alleys. The household distribution is mostly dense, while some are scattered. The overall socio-economic status of the population is between the upper middle and lower economic strata. There are various types of houses in this cluster, but two-storey houses are predominant and their structures are in good condition. The mean distance of nearest houses is about 2.83 meters. The public spaces in this community include one temple, one school, and one children field. All households are provided with a public water supply and daily garbage collection services.

#### **4.1.12 Cluster 12: Pak Nam Jolo**

Pak Nam Jolo is a sub-urban residential mixed with commercial region, with a low degree of dengue transmission. This community is situated alongside the Bangpakong River and has only one main paved road with connecting alleys. The households are densely situated along both sides of the main street, while scattered ones are found around the alleys. The socio-economic status of inhabitants falls between the upper middle and lower economic strata. There are various types of houses in this cluster, with the one storey houses being predominant. Overall, the housing structures are in good condition. The mean distance of nearest houses is about 3.06 meters. There is only one public space, which is a gasoline station located in the area. Nevertheless, this community is situated not far from the community hospital and the sub-district municipality. All residents are provided with electricity, a public water supply, and daily garbage collection services on a regular basis.

A

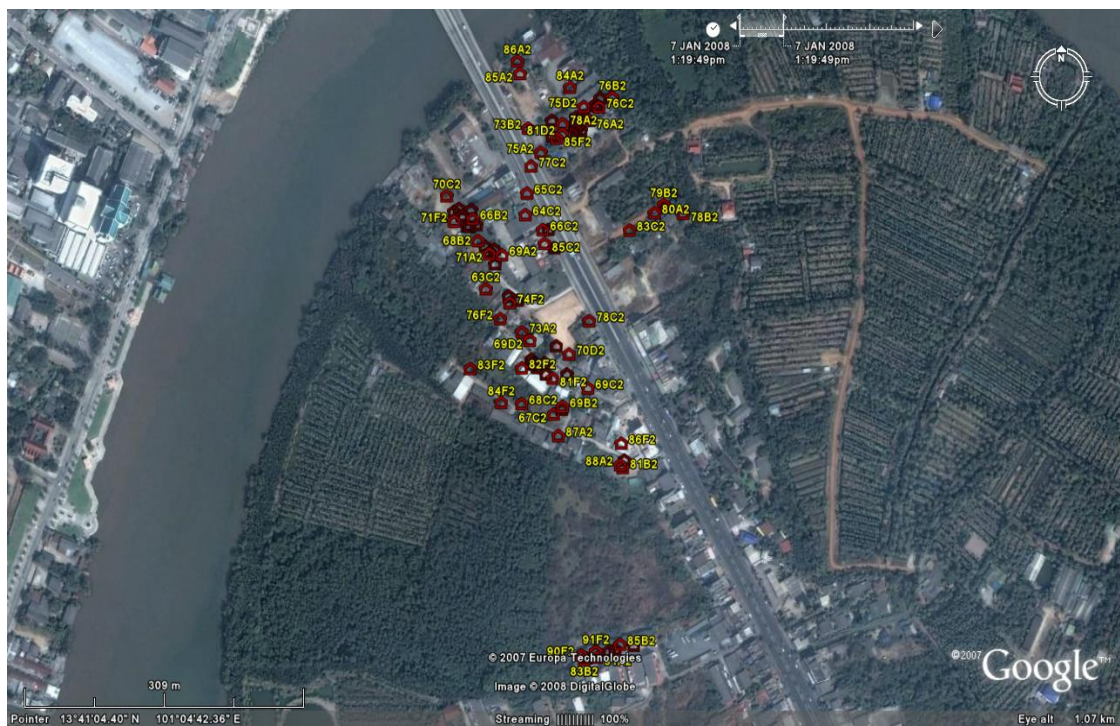


B



**Figure 11** Cluster No.1 (Soi Li-Ke): The ecotope of residential mixed with commercial area and DENPURA with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

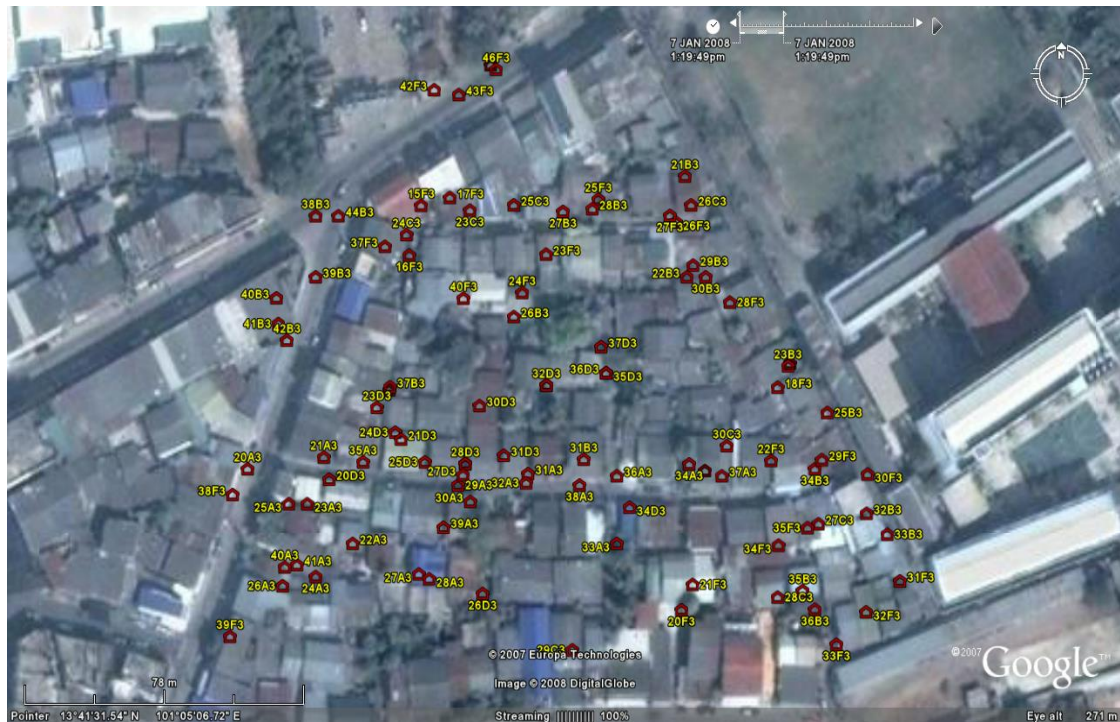


B



**Figure 12** Cluster No.2 (Ta Kham): The ecotope of residential mixed with commercial area and DENPURA with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

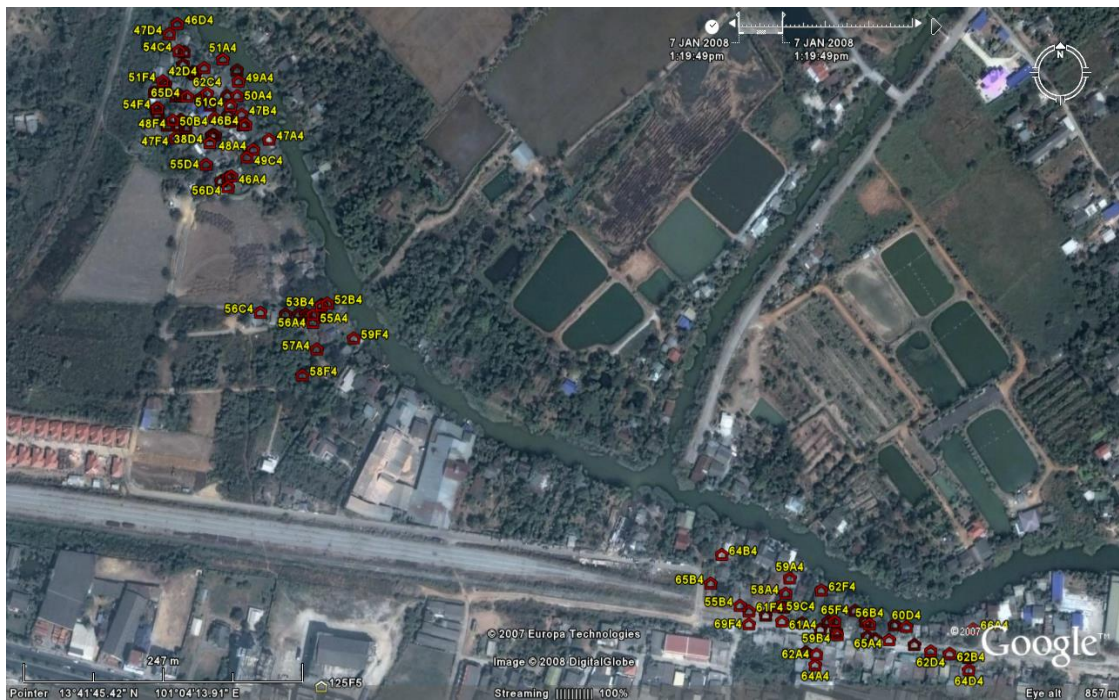


B



**Figure 13** Cluster No.3 (Wannaying): The ecotope of densely populated urban residential area (DENPURA) with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

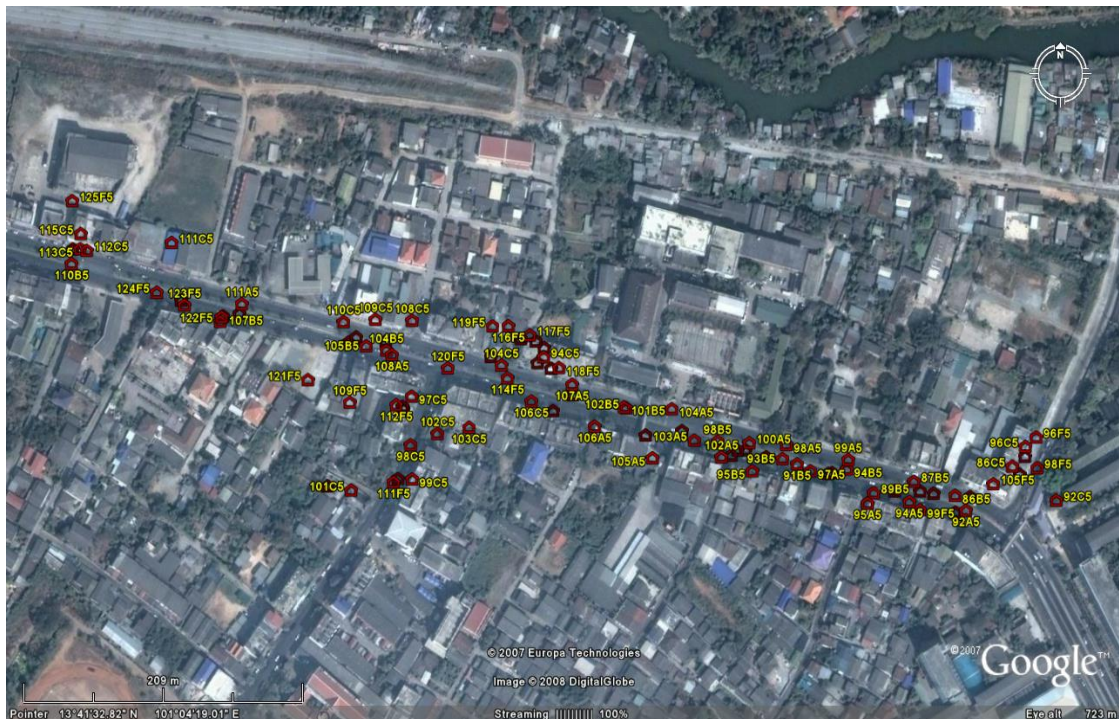


B



**Figure 14** Cluster No.4 (Sapandam): The ecotope of densely populated urban residential area (DENPURA) with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

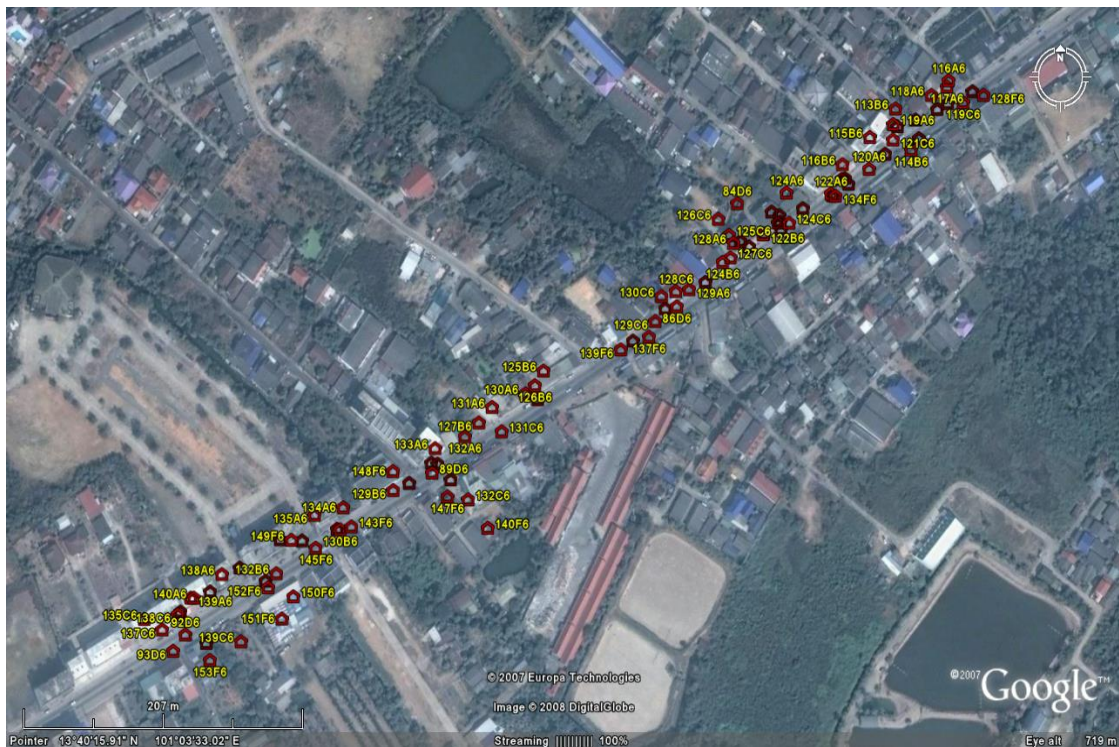


B



**Figure 15** Cluster No.5 (Mahajakapad): The ecotope of commercial area with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A



B



**Figure 16** Cluster No.6 (Tepkunakorn): The ecotope of commercial area with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

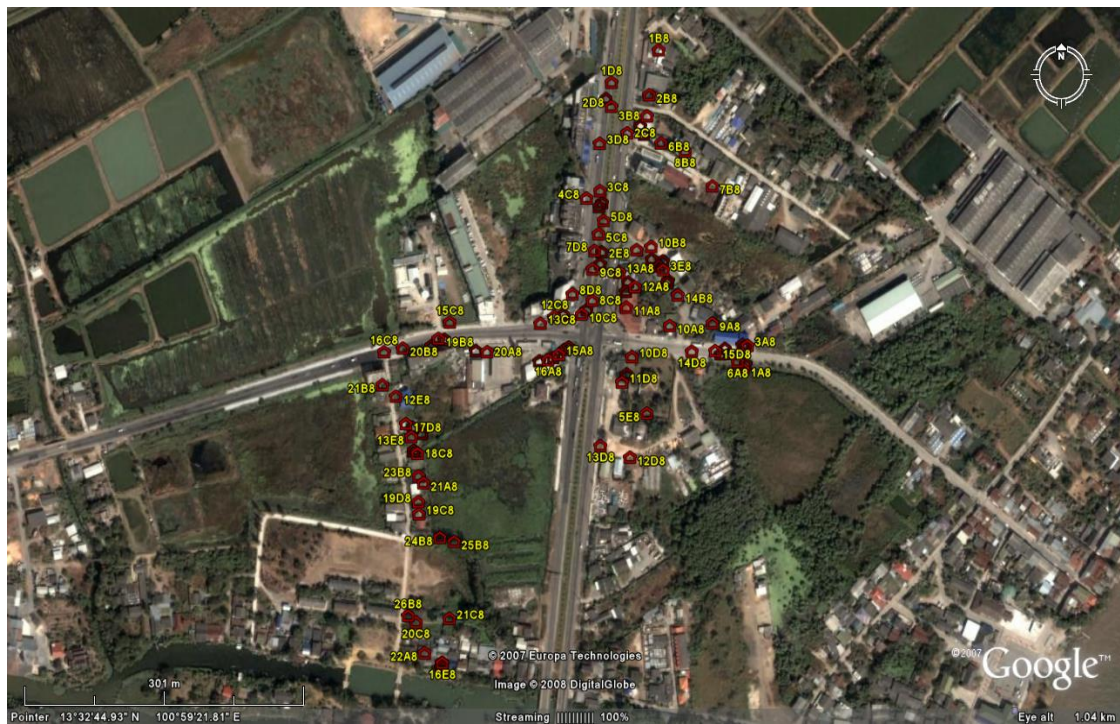


B



**Figure 17** Cluster No.7 (Burapa City): The ecotope of residential mixed with commercial area with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

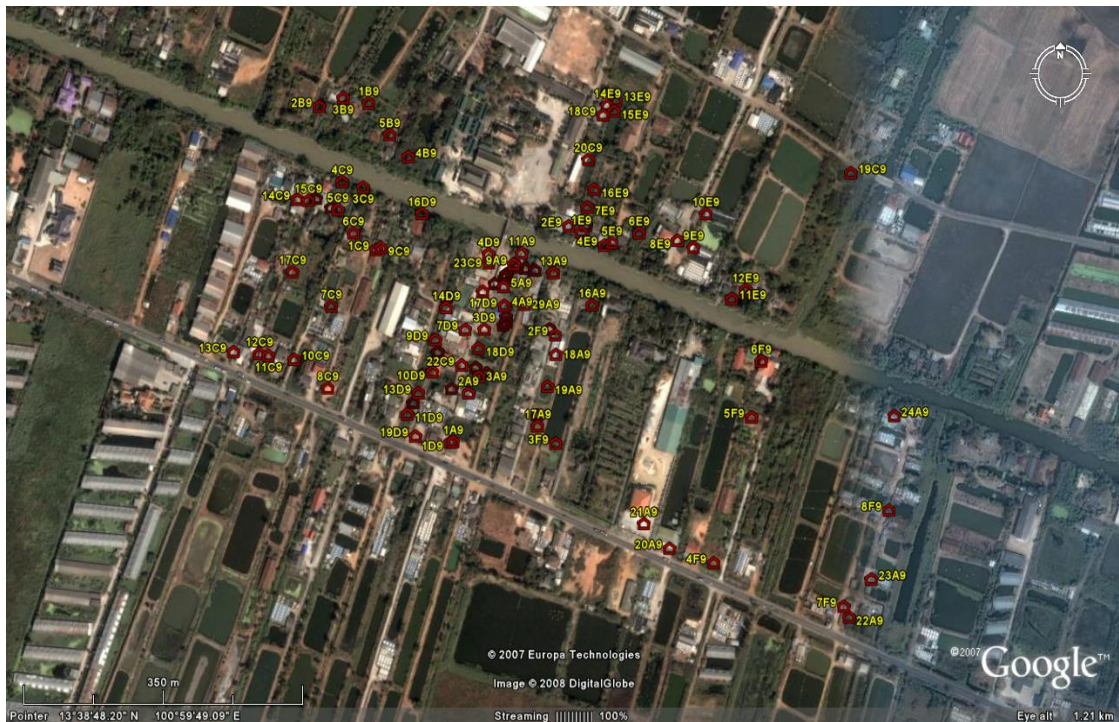


B



**Figure 18** Cluster No.8 (Ta Sa-arn): The ecotope of residential mixed with commercial area with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

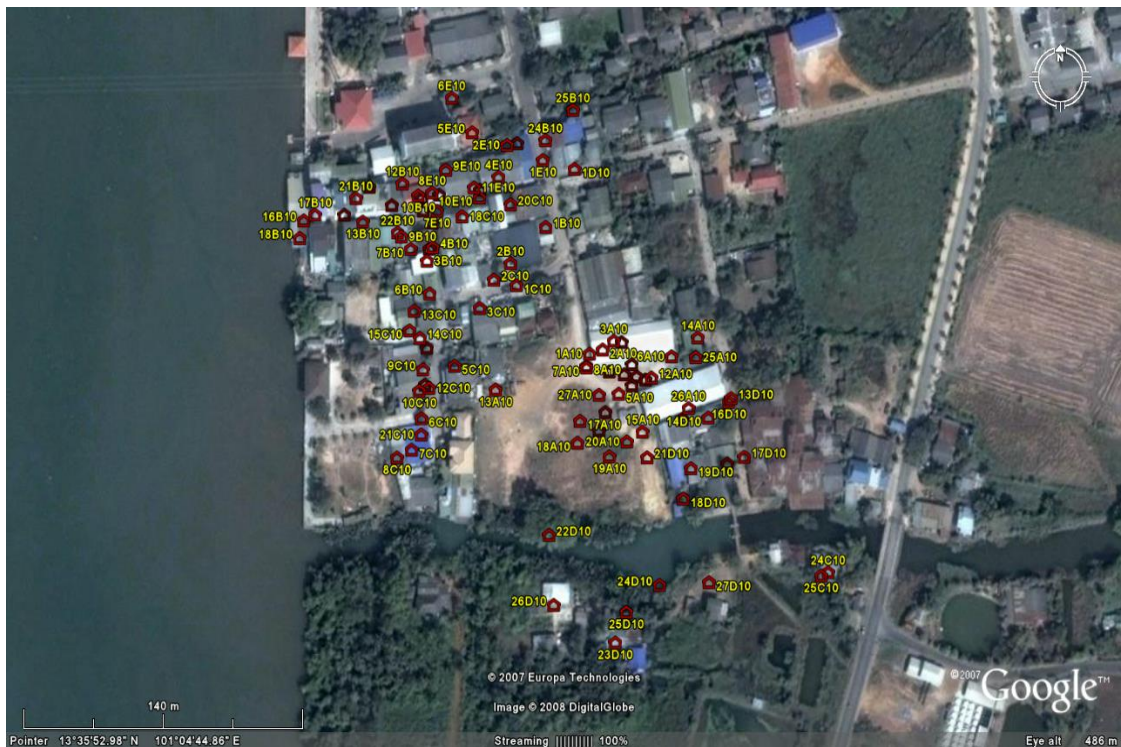


B



**Figure 19** Cluster No.9 (Tepparachbureerom): The ecotope of residential mixed with commercial area with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

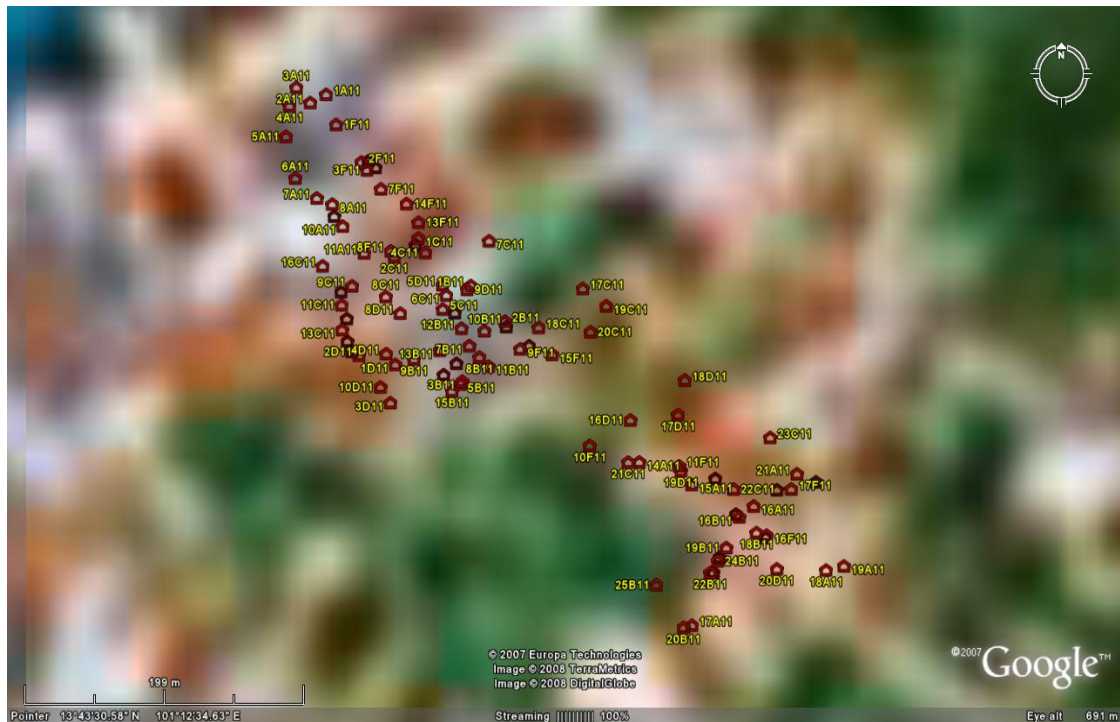


B



**Figure 20** Cluster No.10 (Ban Sanamjan): The ecotope of residential mixed with commercial area with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A

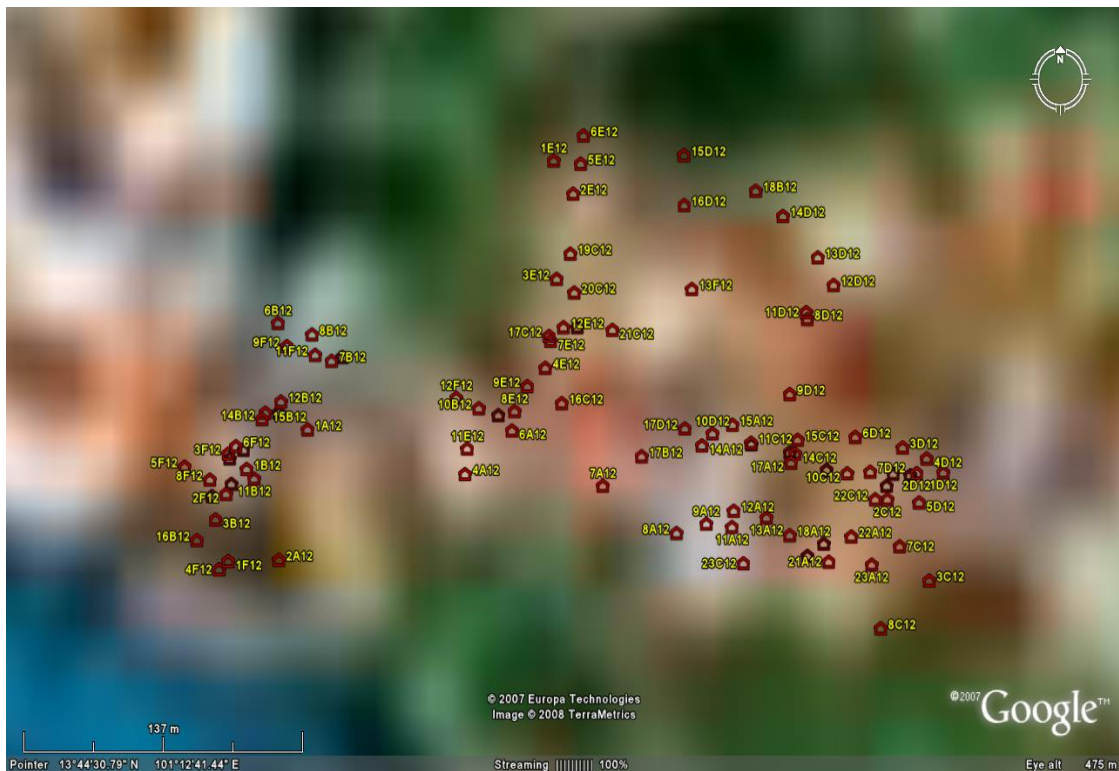


B



**Figure 21** Cluster No.11 (Lung Wat Jaeng): The ecotope of residential mixed with commercial area with a high degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

A



B



**Figure 22** Cluster No.12 (Pak Num Jolo): The ecotope of residential mixed with commercial area with a low degree of dengue transmission. Google Earth map (A) and photograph (B) show the household distribution and cluster environment.

## 4.2 Ecological Investigations

A set of ecological determinants as risk factors were examined for dengue case history and entomological index (Table 2). Both the urban area and semi-urban area experienced the same dengue history. Settings with a high degree of dengue transmission indicated odds ratio (OR) significantly higher than areas with a low degree of dengue transmission. Among the 4 different ecotopes within urban and semi-urban areas, only the ecotope of residential mixed with commercial area and DENPURA had a significant OR of 2.180. When further analyzed for individual ecotopes in high and low dengue transmission areas, this ecotope showed a case history with a 4-fold increase in the risk of dengue with significance.

In similar fashion, the pupal-demographic index as a principal outcome of influencing ecologic factors was predicted using the same antecedent ecotopes. Determinants both of urbanization and the degree of dengue transmission did not estimate the risk of houses with positive breeding containers. In contrast to dengue case history, there was no significant difference in the pupa-demographic index among the 4 ecotopes. For an analysis of individual ecotopes in high and low dengue transmission areas, ecotopes associated with pupa density were tested. In high transmission areas, the ecotope of residential mixed with commercial area alone showed negative association with the pupa-demographic index. Conversely, two ecotopes of residential mixed with commercial area and DENPURA and residential mixed with commercial area demonstrated positive significant ORs of 2.524 and 2.136, respectively, in a low dengue transmission setting.

**Table 2** Risk factors of ecotope characteristics for dengue transmission and vector abundance in urban and semi-urban settings.

Characteristics	Household Number		Dengue case history <sup>a</sup>		OR	P-value	Pupal-demographic <sup>b</sup>		OR	P-value
	Yes	No	Yes	No			Positive	Negative		
<b>Urbanization</b>										
Semi-urban	600	518 (86.3)	82 (13.7)	518 (86.3)	1	1	75 (12.5)	525 (87.5)	1	
Urban	600	518 (86.3)	82 (13.7)	518 (86.3)	1.000	1.000	88 (14.7)	512 (85.3)	1.203	0.273
<b>Degree of dengue transmission</b>										
Low	600	543 (90.5)	57 (9.5)	543 (90.5)	1	1	81 (13.6)	519 (86.5)	1	
High	600	493 (82.2)	107 (17.8)	493 (82.2)	2.068	<0.001	82 (13.7)	518 (86.3)	1.014	0.933
<b>Urban</b>										
DENPURA	200	180 (90.0)	20 (10.0)	180 (90.0)	1	1	28 (14.0)	172 (86.0)	1	
Commercial area	200	177 (88.5)	23 (11.5)	177 (88.5)	1.169	0.628	24 (12.0)	176 (88.0)	0.838	0.552
Residential mixed with commercial area and DENPURA (RCDENPURA)	200	161 (80.5)	39 (19.5)	161 (80.5)	2.180	0.007	36 (18.0)	164 (82.0)	1.348	0.275
<b>Semi-urban</b>										
Residential mixed with commercial area	600	518 (86.3)	82 (13.7)	518 (86.3)	1.425	0.178	75 (12.5)	525 (87.5)	0.878	0.583
<b>High dengue transmission</b>										
DENPURA	100	89 (89.0)	11 (11.0)	89 (89.0)	1	1	20 (20.0)	80 (80.0)	1	
Commercial area	100	89 (89.0)	11 (11.0)	89 (89.0)	1.000	1.000	16 (16.0)	84 (84.0)	0.762	0.462
RCDENPURA	100	69 (69.0)	31 (31.0)	69 (69.0)	3.635	0.001	18 (18.0)	82 (82.0)	0.878	0.718
Residential mixed with commercial area	300	246 (82.0)	54 (18.0)	246 (82.0)	1.776	0.100	28 (9.3)	272 (90.7)	0.412	0.004
<b>Low dengue transmission</b>										
DENPURA	100	91 (91.0)	9 (9.0)	91 (91.0)	1	1	8 (8.0)	92 (92.0)		
Commercial area	100	88 (88.0)	12 (12.0)	88 (88.0)	1.379	0.489	8 (8.0)	92 (92.0)	1.000	1.000
RCDENPURA	100	92 (92.0)	8 (8.0)	92 (92.0)	0.879	0.800	18 (18.0)	82 (82.0)	2.524	0.036
Residential mixed with commercial area	300	272 (90.7)	28 (9.3)	272 (90.7)	1.041	0.921	47 (15.7)	253 (84.3)	2.136	0.054

<sup>a</sup> Number of houses with past dengue cases, <sup>b</sup> Number of houses with positive breeding containers

## **4.3 Biological Implications**

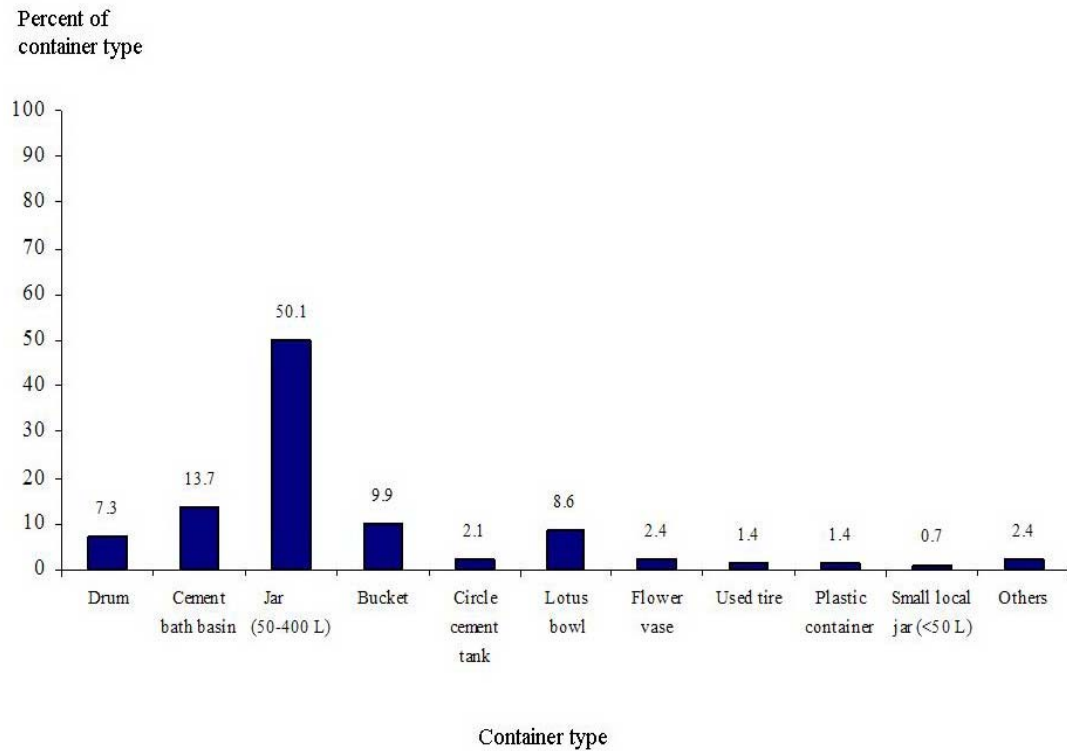
### **4.3.1 Evaluation of *Aedes* vector abundance and relevant environmental factors**

*Aedes* larvae and pupae, together with the mosquito's environmental breeding sites, were inspected in and around the entire 1,200 households of the study area during the wet and dry seasons. The container types, their social use, and productivity in terms of larva and pupa density within these 2 seasonal periods are discussed in the following sections.

#### **4.3.1.1 Entomological investigation in the wet season**

Of the 1,200 premises inspected in the study site during the wet season, 1,194 houses were found to have at least one water-holding container. A total of 7,804 potential containers were observed and classified into 11 categories, as shown in Figure 23. From all the containers evaluated, a majority of them (50.1 %) were 50-400-L jars, followed by cement bath basins (13.7%), buckets (9.9%), lotus bowls (8.6%), and drums (7.3%). The other categories—including flower vases, circle cement tanks, used tires, plastic containers, < 50-L small local jars, and miscellaneous items—accounted for 10.4% of the container categories.

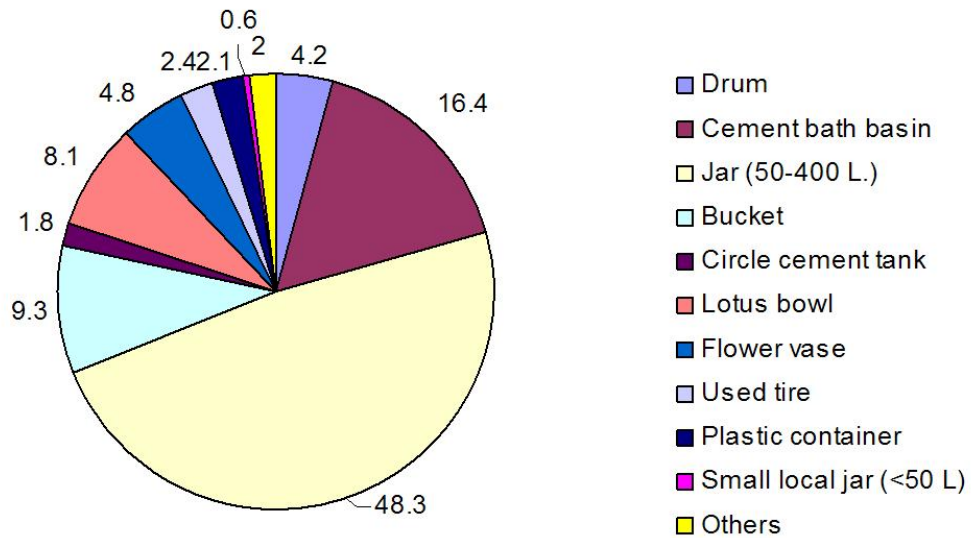
Figures 24-35 individually detail more about the percentage contributions to container types as *Aedes* breeding sites among the 12 examined clusters surveyed during the wet season.



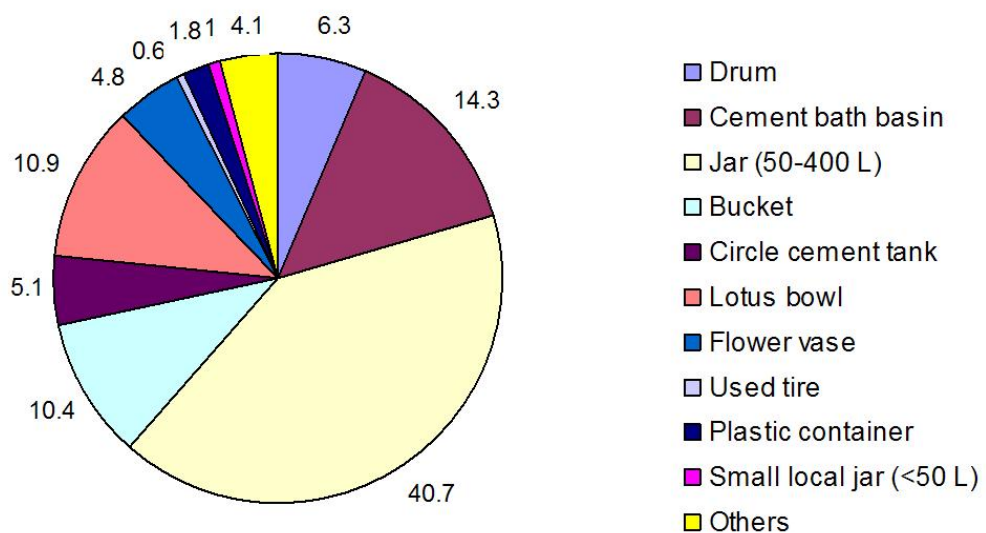
**Figure 23** Percentage contributions to container types observed in the 12 study clusters during the wet season survey.

For cluster no. 1, a majority of containers (48.3 %) were 50-400-L jars, followed by cement bath basins (16.4%), buckets (9.3%), lotus bowls (8.1%), and drums (4.2%). A small portion of the others—including flower vases, circle cement tanks, used tires, plastic containers, < 50-L small local jars, and other categories—constituted 13.7% (Figure 24). The most common water-bearing containers in cluster no. 2 were 50-400-L jars (40.7%), cement bath basins (14.3%), lotus bowls (10.9%), buckets (10.4%), drums (6.3%), cement circle tanks (5.1%), and flower vases (4.8%), respectively, while the other 4 categories comprised only 7.5% (Figure 25). A majority of containers observed in cluster no. 3 were 50-400-L jars (62.1%), cement bath basins (14.9%), buckets (7.7%), and lotus bowls (5.3%), whereas the other containers accounted for 10.0% (Figure 26). The most frequent potential containers discovered in cluster no. 4 were 50-400-L jars (74.2%), followed by buckets (9.1%), and the remainder made up 16.7% (Figure 27). The percentage contributions to container types in cluster no. 5 were 32.6% cement bath basins, 21.3% buckets, 17.9% 50-400-L

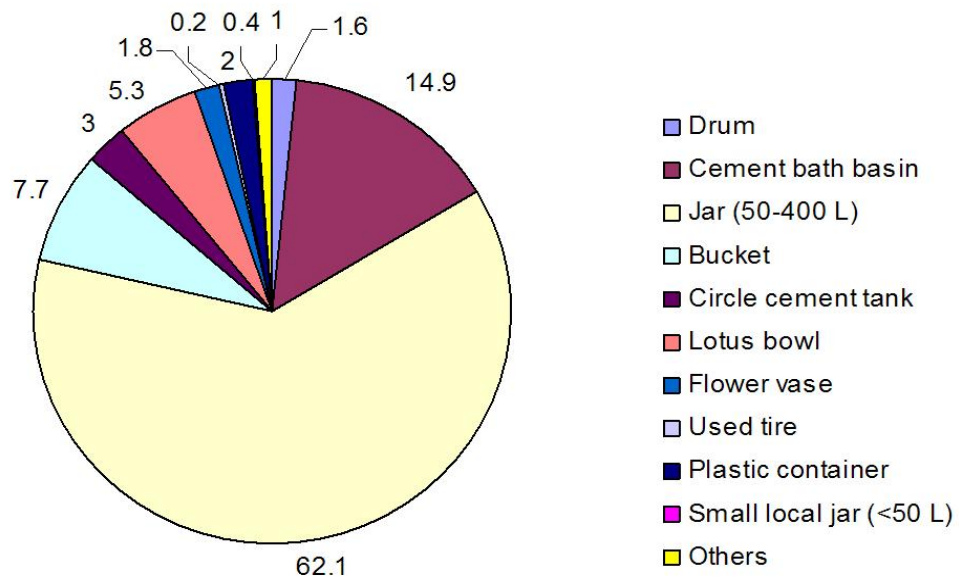
jars, 10.6% lotus bowls, and 17.6% other categories (Figure 28). The surveyed data of cluster no. 6 disclosed 26.4% cement bath basins, 21.7% lotus bowls, 16.0% buckets, 13.6% 50-400-L jars, 12.0% drums, and 10.3% other containers (Figure 29). For cluster no. 7, the most frequent type of receptacle was drums (26.7%), followed by buckets (18.6%), lotus bowls (18.1%), and 50-400-L jars (15.8%), while miscellaneous containers constituted 20.8% (Figure 30). For cluster no. 8, the first four frequent container types included 50-400-L jars (27.4%), cement bath basins (20.2%), lotus bowls (13.7%), and buckets (11.0%), whereas the other categories accounted for 27.7% (Figure 31). The percentage contributions to receptacle types in cluster no. 9 were 66.1% 50-400-L jars, 11.5% cement bath basins, 5.9% drums, 5.6% lotus bowls, and 20.9% for the remainder (Figure 32). The data obtained from cluster no. 10 showed 57.1% 50-400-L jars, 13.1% drums, 10.9% cement bath basins, 7.8% buckets, 5.5% lotus bowls, and 5.6% other categories (Figure 33). For cluster no. 11, the majority of container types were 50-400-L jars (46.4%), cement bath basins (13.5%), buckets (11.6%), and lotus bowls (8.4%), whereas the rest made up 20.1% (Figure 34). For cluster no. 12, the four major container types were 50-400-L jars (62.7%), cement bath basins (10.6%), buckets (8.6%), and lotus bowls (6.4%), while the other categories were responsible for 11.7% (Figure 35).



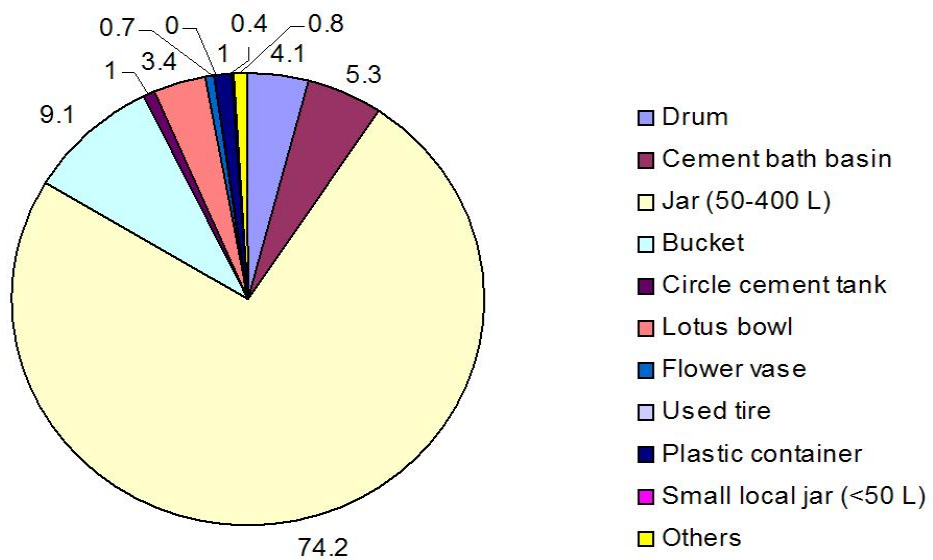
**Figure 24** Percentage contributions to container types observed in cluster no. 1 during the wet season survey.



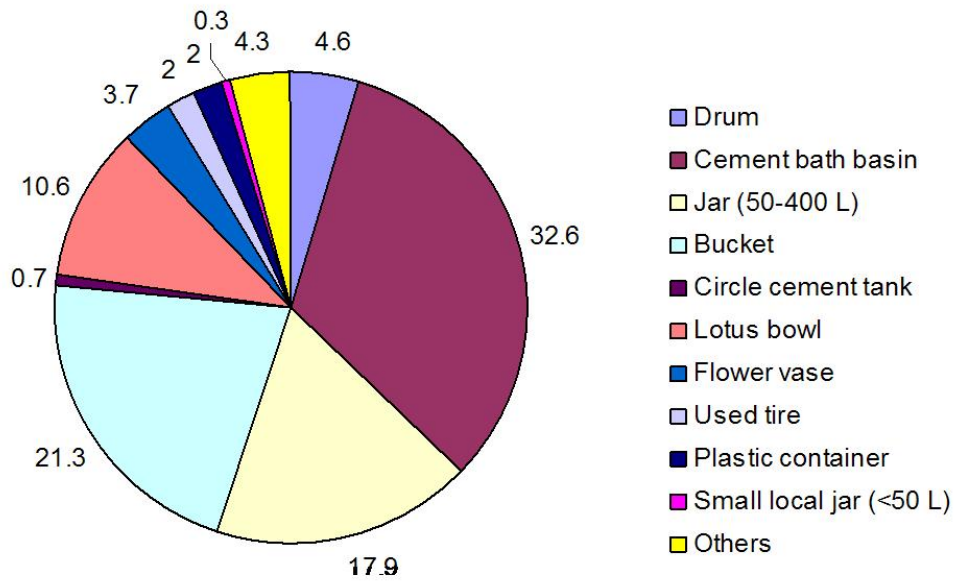
**Figure 25** Percentage contributions to container types observed in cluster no. 2 during the wet season survey.



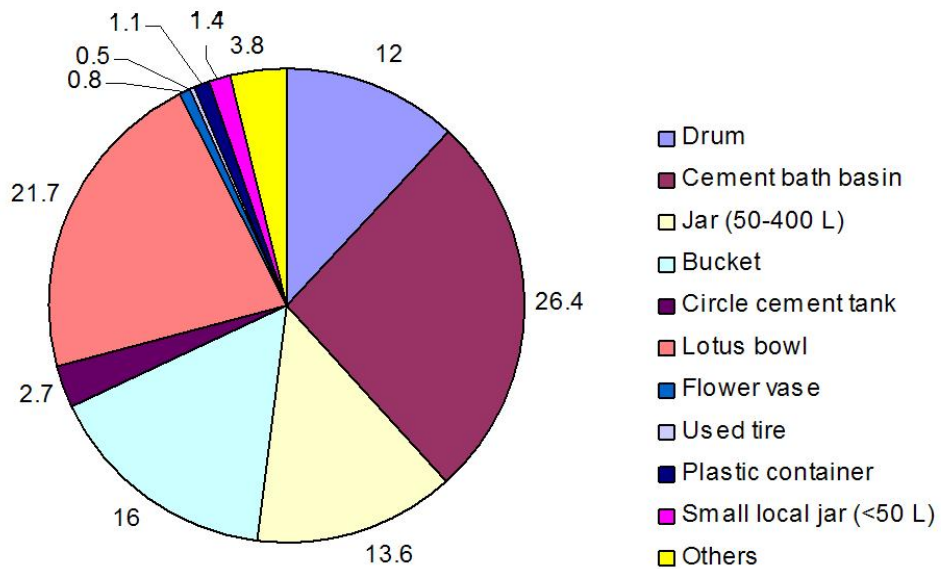
**Figure 26** Percentage contributions to container types observed in cluster no. 3 during the wet season survey.



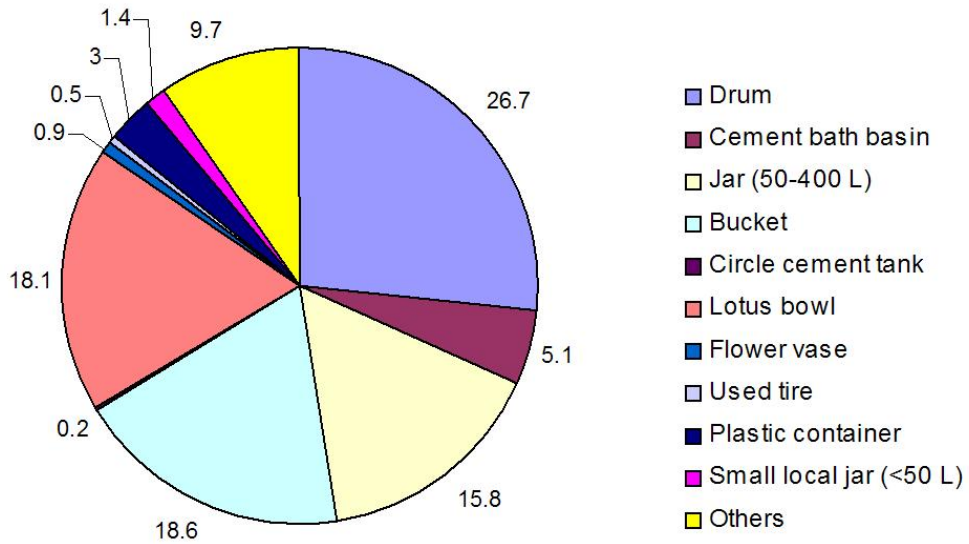
**Figure 27** Percentage contributions to container types observed in cluster no. 4 during the wet season survey.



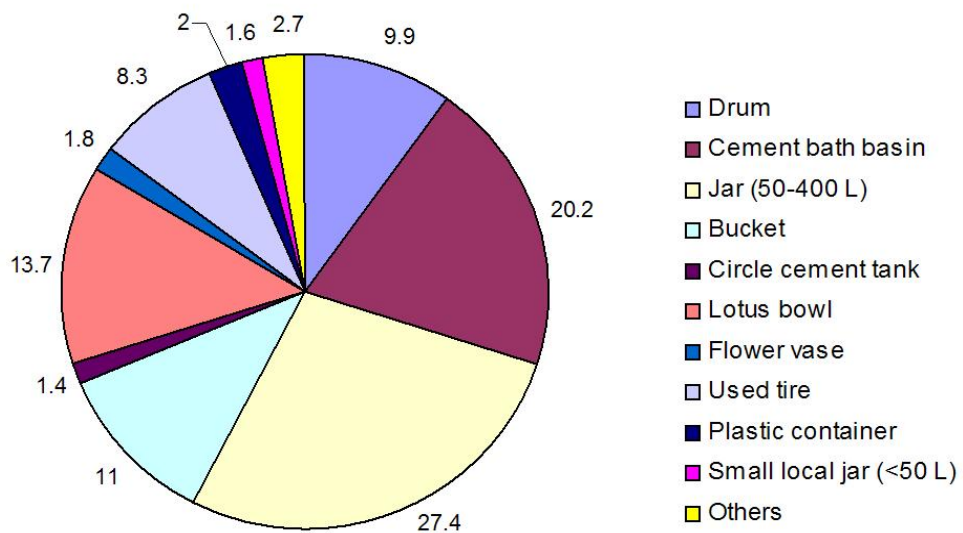
**Figure 28** Percentage contributions to container types observed in cluster no. 5 during the wet season survey.



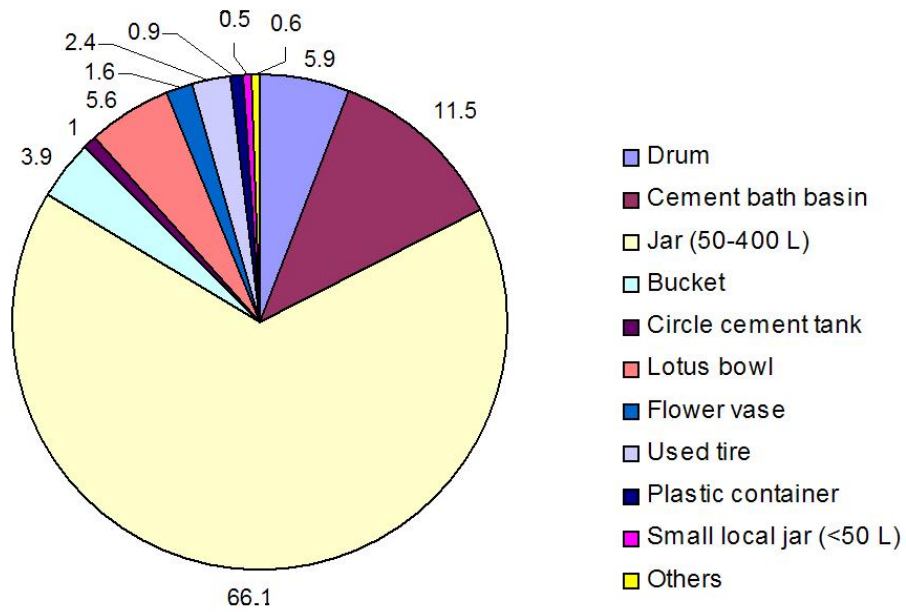
**Figure 29** Percentage contributions to container types observed in cluster no. 6 during the wet season survey.



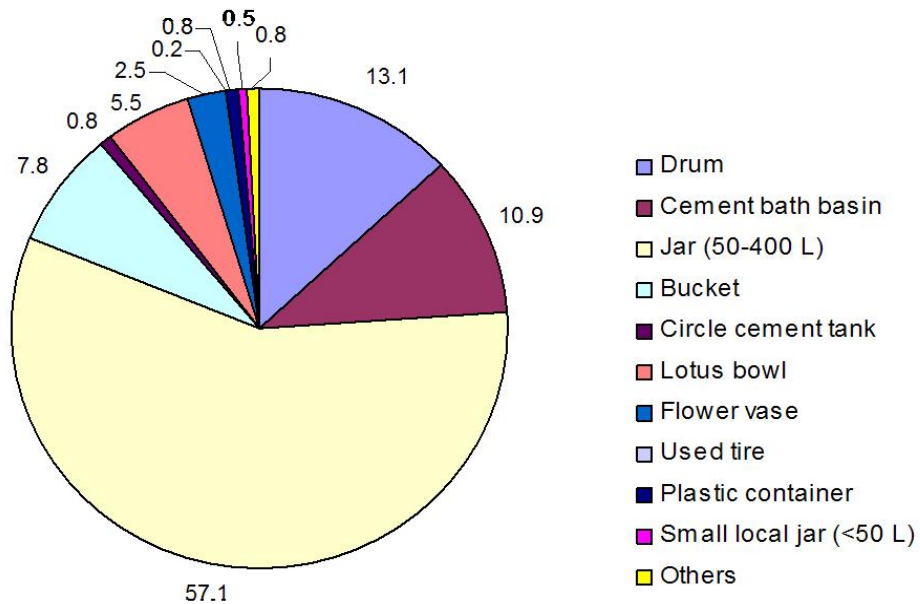
**Figure 30** Percentage contributions to container types observed in cluster no. 7 during the wet season survey.



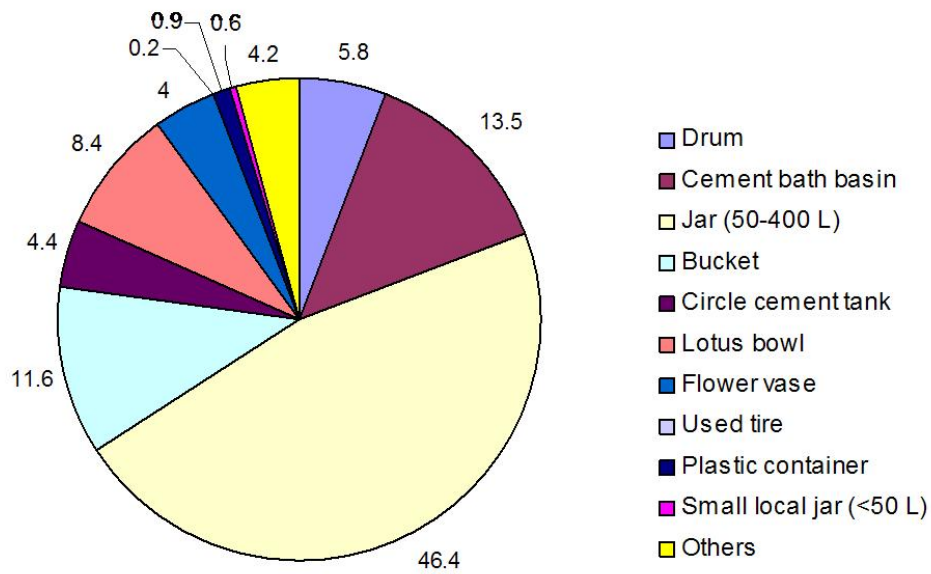
**Figure 31** Percentage contributions to container types observed in cluster no. 8 during the wet season survey.



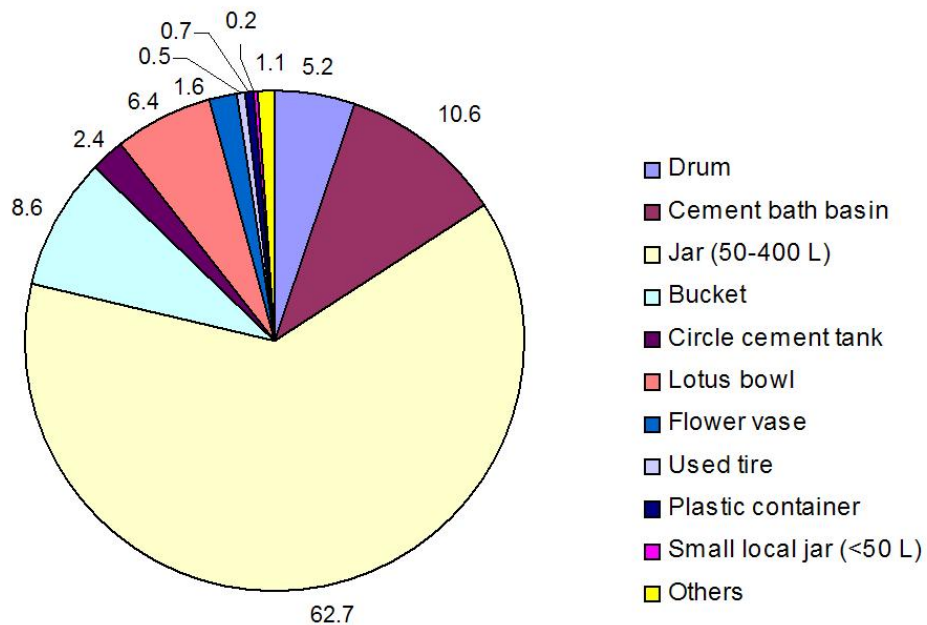
**Figure 32** Percentage contributions to container types observed in cluster no. 9 during the wet season survey.



**Figure 33** Percentage contributions to container types observed in cluster no. 10 during the wet season survey.



**Figure 34** Percentage contributions to container types observed in cluster no. 11 during the wet season survey.



**Figure 35** Percentage contributions to container types observed in cluster no. 12 during the wet season survey.

There were 7,804 water-holding containers observed among 12 study clusters during the wet season survey, the numbers of which were individually distributed among 4 ecotopes (RCDENPURA, DENPURA, C, and RC) in sums of 1,393, 1,658, 669, and 4,084, respectively.

In accordance with individual chi-square tests, results of associations among container characteristics, as well as their social use in relation to 4 different ecotopes, are presented in Table 3. The difference of 4 ecotope types were significantly associated with the type of container ( $P < 0.001$ ), water type ( $P < 0.001$ ), position under vegetation ( $P < 0.001$ ), location ( $P < 0.001$ ), usage in the past 7 days ( $P < 0.001$ ), shade ( $P < 0.001$ ), intervention ( $P < 0.001$ ), and cover ( $P < 0.001$ ). In addition, four individual ecotopes were further investigated to see whether there was an association between the presence of *Aedes* immatures in a habitat and container characteristics, including their social use (Table 4). From a total of 596 *Aedes* immature-positive containers observed among 12 study clusters during the wet season survey, the numbers were individually distributed among 4 ecotopes (RCDENPURA, DENPURA, C, and RC) in sums of 146, 95, 73, and 282, respectively.

Out of the 4 ecotopes individually tested, only 2 (RCDENPURA and RC) demonstrated a significant association between the presence of mosquito immatures and container type, both with  $P < 0.001$ . The water type was strongly associated with the presence of *Aedes* immatures only for the RCDENPURA and DENPURA ecotopes, with P-values of 0.001 and 0.002, respectively. However, there was no association between the immature-positive containers and their position under vegetation for all ecotopes. The association of the number of immature-positive containers among individual ecotopes was made with 3 other variables: location, usage in the past 7 days, and shade. As for location, only the RCDENPURA and RC ecotopes exhibited a significant association with the number of immature-positive containers, with  $P = 0.008$  and  $P = 0.0039$ , respectively, while there was a strong association between usage in the past 7 days and the presence of *Aedes* immatures only for the ecotope of RCDENPURA ( $P = 0.005$ ). Unlike the location and usage in the past 7 days, shade was associated with the number of immature-positive containers for up to 3 ecotopes: RCDENPURA ( $P = 0.002$ ), DENPURA ( $P = 0.014$ ), and RC ( $P = 0.008$ ). The last two variables statistically examined were intervention and cover, and the results of both

variables demonstrated a significant association with the number of immature-positive containers for all 4 various ecotopes, almost all with  $P < 0.001$ .

**Table 3** Number and percent of container characteristics and their social use in relation to 4 different ecotopes surveyed in the wet season.

Container characteristics and their social use	Number of containers (%)				P-value
	RCDENPURA (N=1,393)	DENPURA (N=1,658)	C (N=669)	RC (N=4,084)	
<b>Type of container</b>					
Drum	74 (5.3)	48 (2.9)	58 (8.7)	392 (9.6)	< 0.001
Cement bath basin	213 (15.3)	166 (10.0)	195 (29.2)	493 (12.1)	
Jar (50-400L)	618 (44.4)	1,132 (68.3)	104 (15.5)	2,053 (50.3)	
Bucket	138 (9.9)	140 (8.4)	123 (18.4)	375 (9.2)	
Circle cement tank	49 (3.5)	32 (1.9)	12 (1.8)	72 (1.8)	
Lotus bowl	133 (9.6)	72 (4.3)	112 (16.7)	352 (8.6)	
Flower vase	67 (4.8)	21 (1.3)	14 (2.1)	85 (2.1)	
Used tire	20 (1.4)	2 (0.1)	8 (1.2)	79 (1.9)	
Plastic container	27 (1.9)	24 (1.5)	10 (1.5)	50 (1.2)	
Small local jar	11 (0.8)	6 (0.4)	6 (0.9)	29 (0.7)	
Others	43 (3.1)	15 (0.9)	27 (4.0)	104 (2.5)	
<b>Water type</b>					
Tap water/Well	1,071 (76.9)	1,063 (64.1)	606 (90.6)	2,490 (61.0)	< 0.001
Rainwater	270 (19.4)	405 (24.4)	47 (7.0)	1,449 (35.5)	
Canal	52 (3.7)	190 (11.5)	16 (2.4)	145 (3.5)	

**Table 3 (Continued)** Number and percent of container characteristics and their social use in relation to 4 different ecotopes surveyed in the wet season.

Container characteristics and their social use	Number of containers (%)				P-value
	RCDENPURA (N=1,393)	DENPURA (N=1,658)	C (N=669)	RCDENPURA (N=1,393)	
<b>Under vegetation</b>					
Fully	93 (6.7)	131 (7.9)	22 (3.3)	332 (8.1)	< 0.001
Partially	96 (6.9)	132 (8.0)	22 (3.3)	339 (8.3)	
Not	1,204 (86.4)	1,395 (84.1)	625 (93.4)	3,413 (83.6)	
<b>Location</b>					
Outside	541 (38.8)	661 (39.9)	73 (10.9)	1,672 (40.9)	< 0.001
Inside	852 (61.2)	997 (60.1)	596 (89.1)	2,412 (59.1)	
<b>Usage in the past 7 days</b>					
Yes	940 (67.5)	1,221 (73.6)	496 (74.1)	2,601 (63.7)	< 0.001
No	453 (32.5)	437 (26.4)	173 (25.9)	1,483 (36.3)	
<b>Shade</b>					
Fully	785 (56.3)	992 (59.8)	572 (85.5)	2,556 (62.6)	< 0.001
Partially	274 (19.7)	240 (14.5)	54 (8.1)	665 (16.3)	
Not	334 (24.0)	426 (25.7)	43 (6.4)	863 (21.1)	
<b>Intervention</b>					
Yes	711 (51.0)	1,019 (61.5)	378 (56.5)	2,721 (66.6)	< 0.001
No	682 (49.0)	639 (38.5)	291 (43.5)	1,363 (33.4)	
<b>Cover</b>					
Preventable	394 (28.3)	794 (47.9)	119 (17.8)	1,730 (42.4)	< 0.001
Partially	159 (11.4)	258 (15.6)	130 (19.4)	550 (13.5)	
Not	840 (60.3)	606 (36.5)	420 (62.8)	1,804 (44.2)	

\*Significantly different ( $P < 0.05$ ) with  $\chi^2$  test for four independent samples.

**Table 4** Number and percent of immature-positive containers in relation to characteristics and their social use among 4 different ecotopes surveyed in the wet season.

Container characteristics and their social use	Number of immature-positive containers (%)			
	RCDENPURA (N=146)	DENPURA (N=95)	C (N=73)	RC (N=282)
<b>Type of container</b>	P < 0.001	N/A	N/A	P < 0.001
Drum	4 (2.7)	3 (3.2)	9 (12.4)	36 (12.8)
Cement bath basin	42 (28.8)	29 (30.5)	32 (43.8)	68 (24.1)
Jar (50-400L)	57 (39.0)	40 (42.1)	16 (21.4)	78 (27.7)
Bucket	6 (4.1)	6 (6.3)	9 (12.3)	34 (12.1)
Circle cement tank	3 (2.1)	1 (1.1)	2 (2.7)	2 (0.7)
Lotus bowl	18 (12.3)	11 (11.6)	1 (1.4)	10 (3.5)
Flower vase	2 (1.4)	1 (1.1)	0 (0)	2 (0.7)
Used tire	8 (5.5)	0 (0)	0 (0)	27 (9.6)
Plastic container	3 (2.1)	4 (4.2)	2 (2.7)	8 (2.8)
Small local jar	1 (0.6)	0 (0)	0 (0)	5 (1.8)
Others	2 (1.4)	0 (0)	2 (2.7)	12 (4.2)
<b>Water type</b>	P = 0.001	P = 0.002	P = 0.151	P = 0.731
Tap water/Well	94 (64.4)	77 (81.0)	65 (89.0)	174 (61.7)
Rainwater	45 (30.8)	11 (11.6)	8 (11.0)	96 (34.0)
Canal	7 (4.8)	7 (7.4)	0 (0)	12 (4.3)
<b>Under vegetation</b>	P = 0.183	P = 0.674	N/A	P = 0.132
Fully	15 (10.3)	6 (6.3)	1 (1.4)	27 (9.6)
Partially	10 (6.8)	6 (6.3)	1 (1.4)	31 (11.0)
Not	121 (82.9)	83 (87.4)	71 (97.2)	224 (79.4)

**Table 4 (Continued)** Number and percent of immature-positive containers in relation to characteristics and their social use among 4 different ecotopes surveyed in the wet season.

Container characteristics and their social use	Number of immature-positive containers (%)			
	RCDENPURA (N=146)	DENPURA (N=95)	C (N=73)	RC (N=282)
<b>Location</b>	P = 0.008	P = 0.089	P = 0.989	P = 0.039
Outside	42 (28.8)	30 (31.6)	8 (11.0)	99 (35.1)
Inside	104 (71.2)	65 (68.4)	65 (89.0)	183 (64.9)
<b>Usage in the past 7 days</b>	P = 0.511	P = 0.342	P = 0.005	P = 0.137
Yes	95 (65.1)	66 (69.5)	64 (87.7)	168 (59.6)
No	51 (34.9)	29 (30.5)	9 (12.3)	114 (40.4)
<b>Shade</b>	P = 0.002	P = 0.014	P = 0.395	P = 0.008
Fully	102 (69.9)	70 (73.7)	65 (89.0)	199 (70.6)
Partially	23 (15.7)	11 (11.6)	6 (8.2)	42 (14.9)
Not	21 (14.4)	14 (14.7)	2 (2.8)	41 (14.5)
<b>Intervention</b>	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Yes	36 (24.7)	34 (35.8)	21 (28.8)	93 (33.0)
No	110 (75.3)	61 (64.2)	52 (71.2)	189 (67.0)
<b>Cover</b>	P < 0.001	P < 0.001	P = 0.001	P < 0.001
Preventable	13 (8.9)	6 (6.3)	2 (2.7)	26 (9.2)
Partially	25 (17.1)	22 (23.2)	15 (20.6)	61 (21.6)
Not	108 (74.0)	67 (70.5)	56 (76.7)	195 (69.2)

\*Significantly different ( $P < 0.05$ ) with  $\chi^2$  test for two independent samples.

N/A = Not available for  $\chi^2$  due to the excess of the expected count being less than 5.

Of 1,200 houses distributed in the 12 study clusters and entomologically inspected during the wet season, 1,194 houses were found to have at least one water-filled container, and a total of 365 houses were positive for *Aedes* immatures. A sum of 7,804 containers was observed in the entire area, of which 596 containers were infested, harboring 2,244 *Aedes* immatures. There were 4,160 family members inhabiting the 1,200 houses during the survey. All sets of these entomologic and epidemiologic data were further analyzed to determine dengue transmission risk by applying four entomological indices: House Index, Container Index, Breteau Index, and pupae per person.

Table 5 compares the larval and pupal indices of *Aedes* vectors with regard to 4 various ecotopes. The House Indices of RCDENPURA, DENPURA, C, and RC were 43.50, 28.00, 26.00, and 28.30, respectively, while the Container Indices of those 4 ecotopes were 10.48, 5.72, 26.00, and 28.30, respectively. The Breteau Indices of the same 4 ecotopes were 73.00, 47.50, 36.50, and 47.00, respectively. In addition to these 3 traditional entomological indices, pupae per person (the more effective pupal-demographic index) was used to determine the risk of dengue transmission. The results of pupae per person among the 4 ecotopes showed 0.31, 0.47, 0.33, and 0.42 respectively.

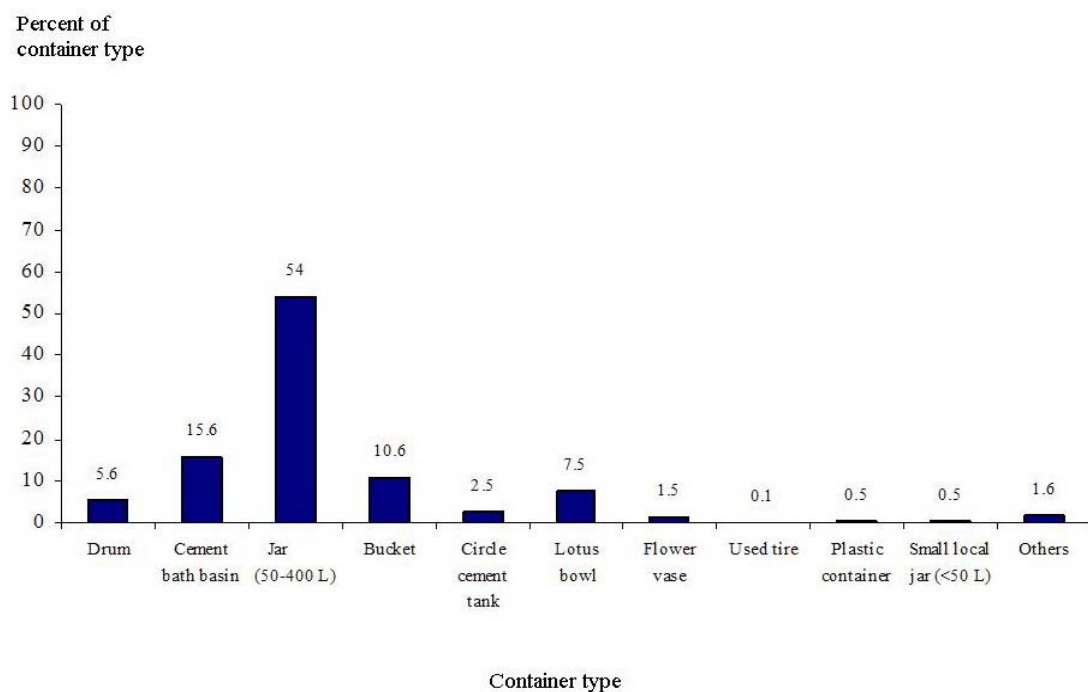
**Table 5** Larval and pupal indices of *Aedes* mosquitoes with respect to 4 different ecotopes surveyed in the wet season.

<b>Larval and Pupal Indices</b>	<b>Residential mixed with commercial and DENPURA</b>	<b>DENPURA</b>	<b>Commercial area</b>	<b>Residential mixed with commercial area</b>
House Index (HI)	43.50	28.00	26.00	28.30
Container Index (CI)	10.48	5.72	26.00	28.30
Breteau Index (BI)	73.00	47.50	36.50	47.00
Pupae per Person (PP)	0.31	0.47	0.33	0.42

### 4.3.1.2 Entomological investigation in the dry season

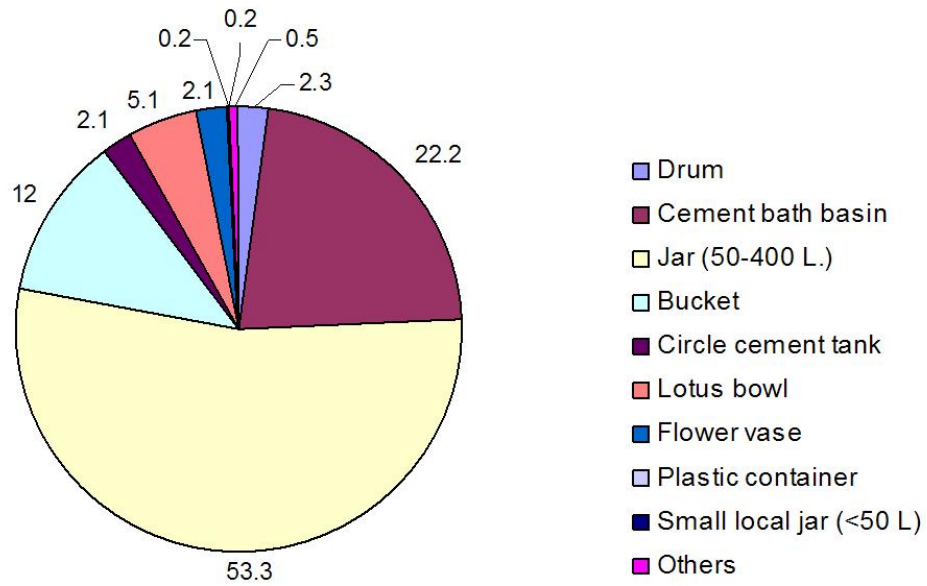
The same 1,200 houses previously examined for entomologic implications during the wet season, were re-inspected in the same manner during the dry season of the following year. Overall, a total of 1,193 premises were found to contain at least one water-bearing container, with a total of 6,836 potential containers being observed during this survey. Similar to the analyses of entomologic datasets in the wet season, the entire 6,836 water-holding containers were categorized into 11 groups, as depicted in Figure 36. Among all categories of containers evaluated, 50-400-L jars were the most frequent habitat observed (54%), followed by cement bath basins (15.6%), buckets (10.6%), lotus bowls (7.5%), and drums (5.6%). The other categories—including circle cement tanks, flower vases, <50-L small local jars, plastic containers, used tires, and other containers—were responsible for 6.7% of all container groups.

Figures 37-48 provide cluster-specific details in terms of the percentage contribution to container types as *Aedes* potential habitats among the 12 clusters re-evaluated during the dry season.

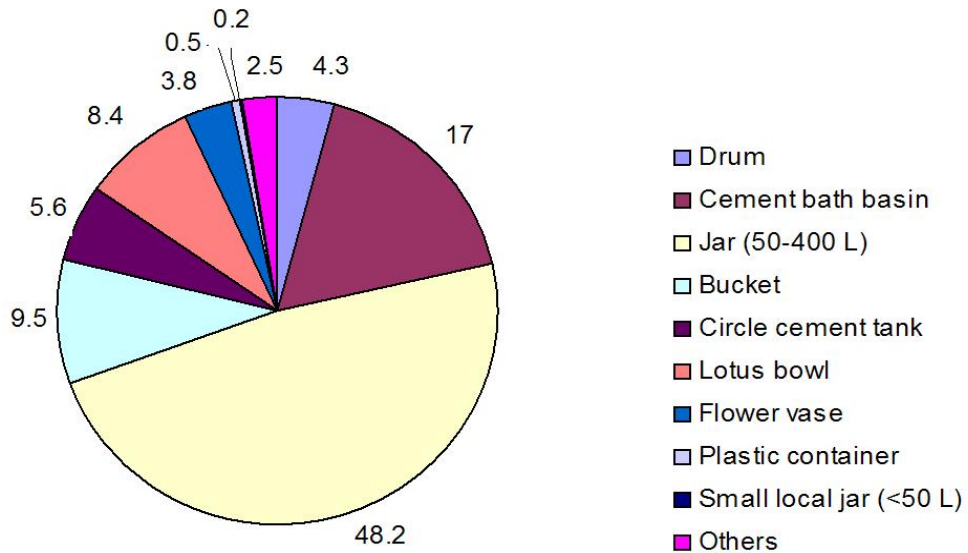


**Figure 36** Percentage contributions to container types observed among the 12 study clusters during the dry season survey.

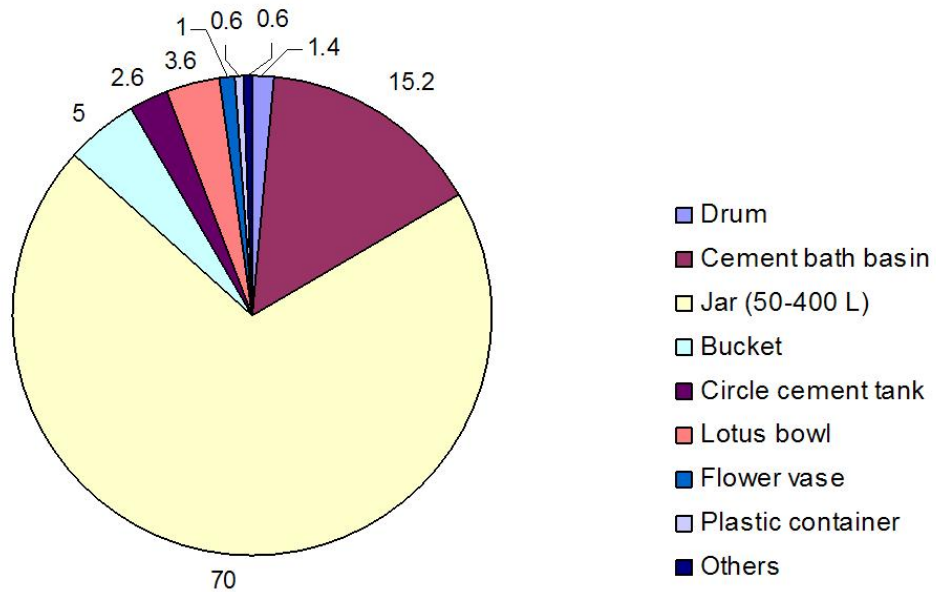
For cluster no. 1, the most common water-filled containers were 50-400-L jars (53.3%), followed by cement bath basins (22.2%), buckets (12%), and lotus bowls (5.1%). A small portion—including drums, flower vases, circle cement tanks, used tires, plastic containers, < 50-L small local jars, and miscellaneous items constituted 7.4% (Figure 37). The largest percentage contributions to container types found in cluster no. 2 were 50-400-L jars (48.2%), cement bath basins (17%), buckets (9.5%), lotus bowls (8.4%), circle cement tanks (5.6%), and drums (4.3%), respectively, while a small remainder comprised 7.0% (Figure 38). A majority of the potential containers observed in cluster no. 3 were 50-400-L jars (70.0%), cement bath basins (15.2%), buckets (5%), and lotus bowls (3.6 %), while a small portion of the other containers accounted for 6.2% (Figure 39). The most frequent potential containers discovered in cluster no. 4 were also 50-400-L jars (81.2%), followed by buckets (7.8%) and drums (3.3%), whereas the remainder made up 7.7% (Figure 40). The percentage contribution to container types in cluster no. 5 were 36.2% cement bath basins, 22.5% 50-400-L jars, 18.8% buckets, 10.3% lotus bowls, 6.6% drums, and 5.6% other miscellaneous items (Figure 41). The surveyed data of cluster no. 6 showed 30.3% cement bath basins, 20.6% lotus bowls, 17.5% buckets, 10.0% 50-400-L jars, 10.0% drums, and 11.6% other containers (Figure 42). For cluster no. 7, the most frequent type of receptacle was buckets (22.3%), followed by drums (22.0%), lotus bowls (19.0%), and 50-400-L jars (16.5%), and cement bath basins (8.2%), while other items constituted 12.0% (Figure 43). For cluster no. 8, the first five frequent receptacle types included 50-400-L jars (43.2%), cement bath basins (23.2%), buckets (12.0%), lotus bowls (8.5%), and drums (8.0%), whereas the other categories accounted for only 5.1% (Figure 44). The percentage contributions to receptacle types in cluster no. 9 were 66.2% 50-400-L jars, 15.0% cement bath basins, 6.0% buckets, 5.0% lotus bowls, 2.9% circle cement tanks, 2.5% drums, and 2.4% the remainder (Figure 45). The data obtained from cluster no. 10 showed 51.5% 50-400-L jars, 14.8% drums, 12.5% buckets, 11.7% cement bath basins, 4.8% lotus bowls, and 4.7% other categories (Figure 46). For cluster no. 11, the majority of container types were 50-400-L jars (47.8 %), cement bath basins (15.0%), buckets (12.1%), and lotus bowls (9.0%), whereas the remaining 7 types made up only 16.1% (Figure 45). For cluster no. 12, the four major container types were 50-400-L jars (62.4%), cement bath basins (11.4%), buckets (10.0%), and lotus bowls (8.7%), while the other 7 categories were merely responsible for 7.5% (Figure 48).



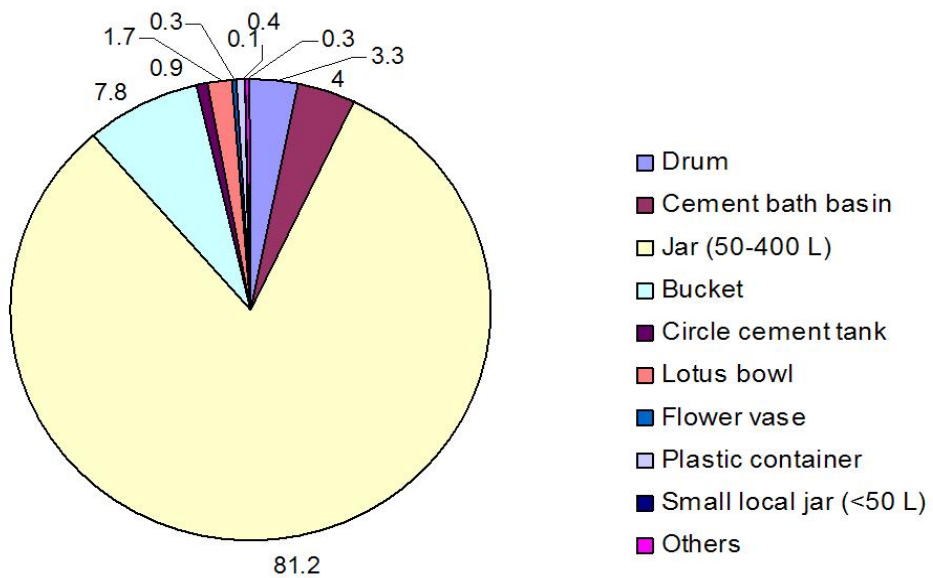
**Figure 37** Percentage contributions to container types observed in cluster no. 1 during the dry season survey.



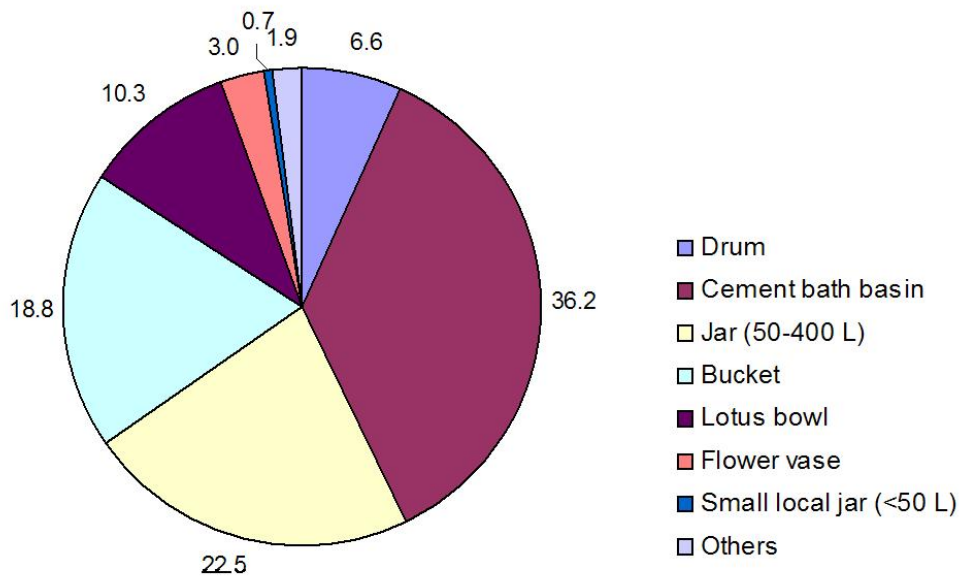
**Figure 38** Percentage contributions to container types observed in cluster no. 2 during the dry season survey.



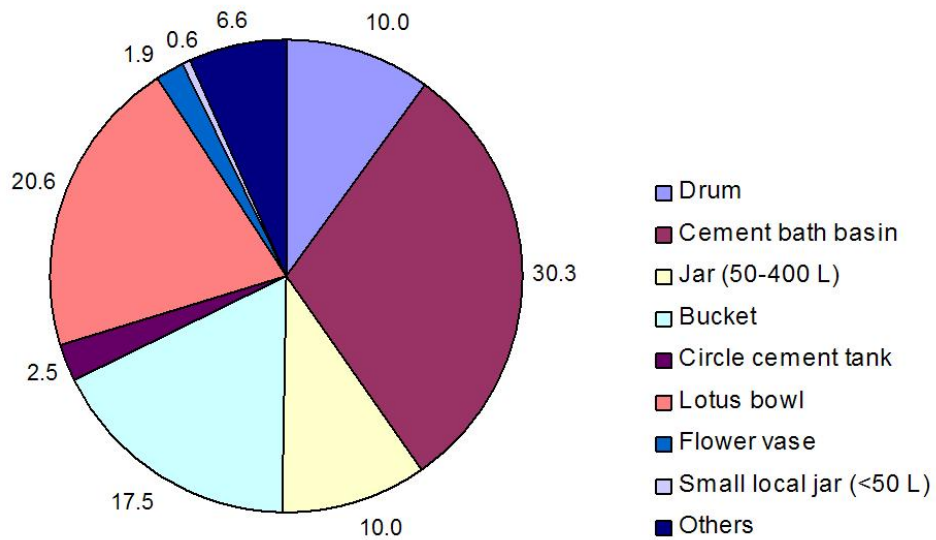
**Figure 39** Percentage contributions to container types observed in cluster no. 3 during the dry season survey.



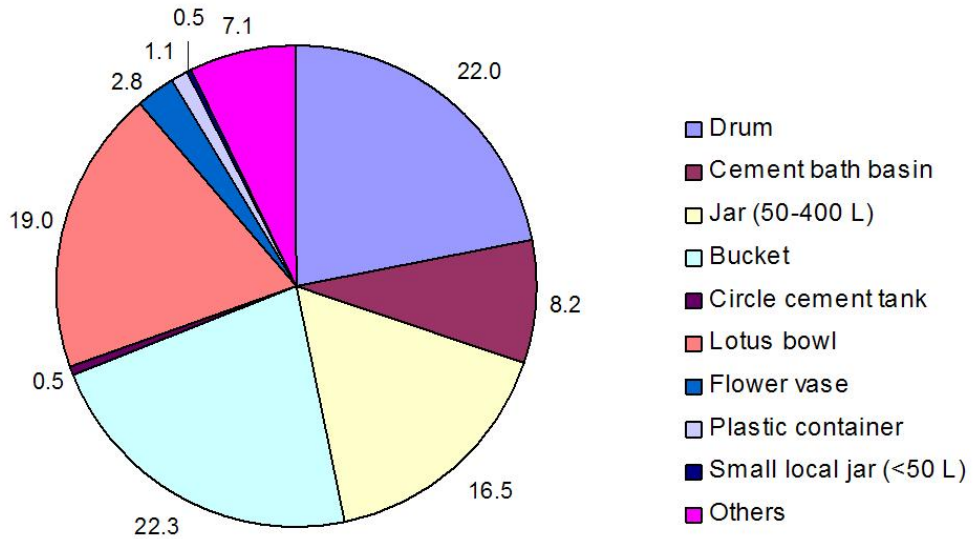
**Figure 40** Percentage contributions to container types observed in cluster no. 4 during the dry season survey.



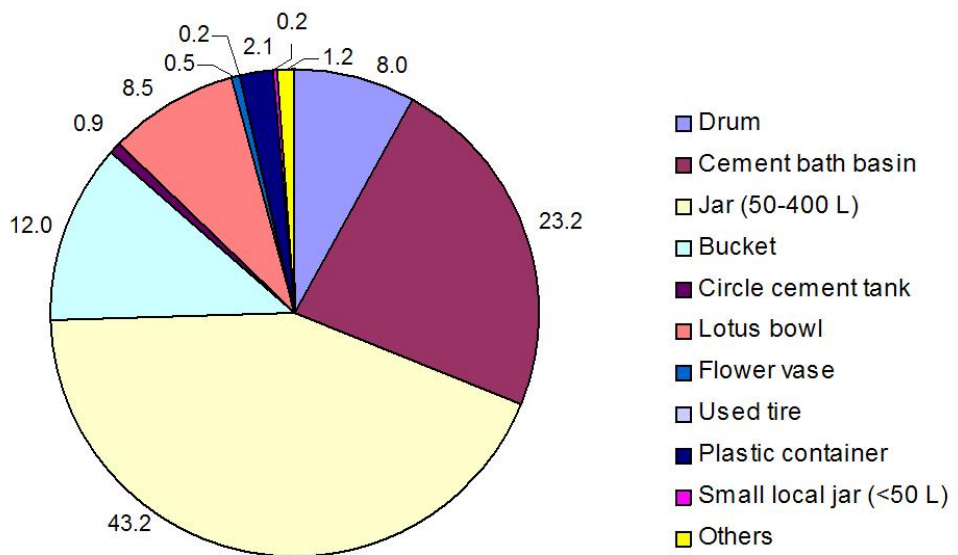
**Figure 41** Percentage contributions to container types observed in cluster no. 5 during the dry season survey.



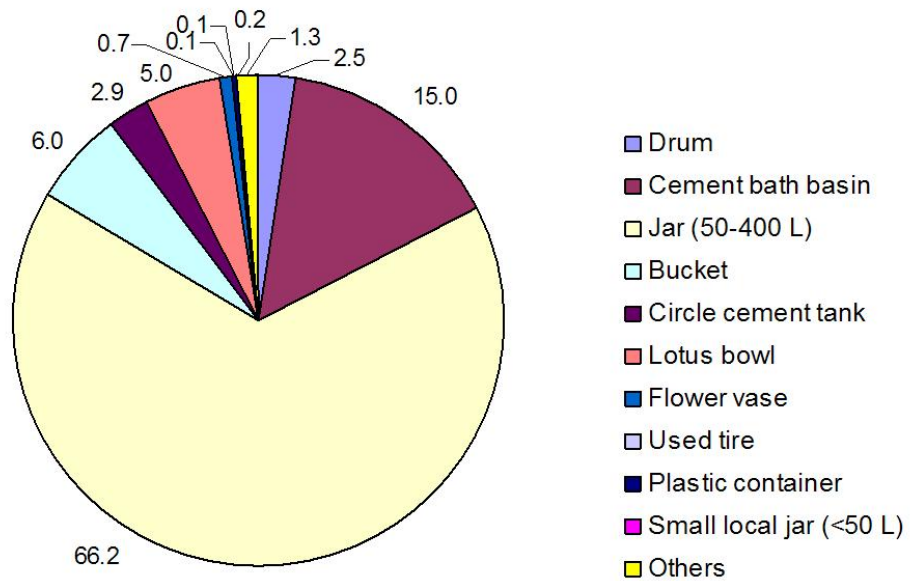
**Figure 42** Percentage contributions to container types observed in cluster no. 6 during the dry season survey.



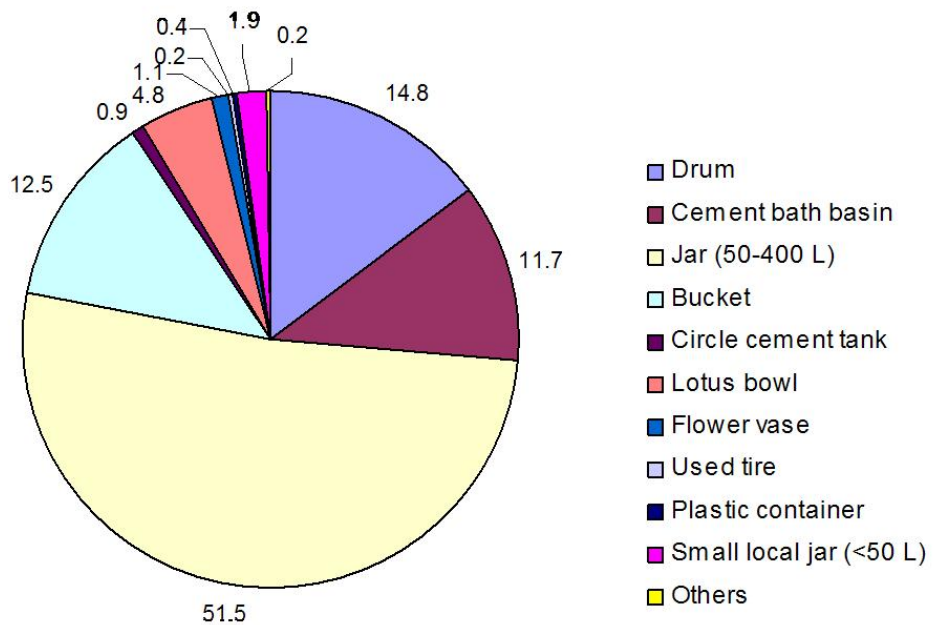
**Figure 43** Percentage contributions to container types observed in cluster no. 7 during the dry season survey.



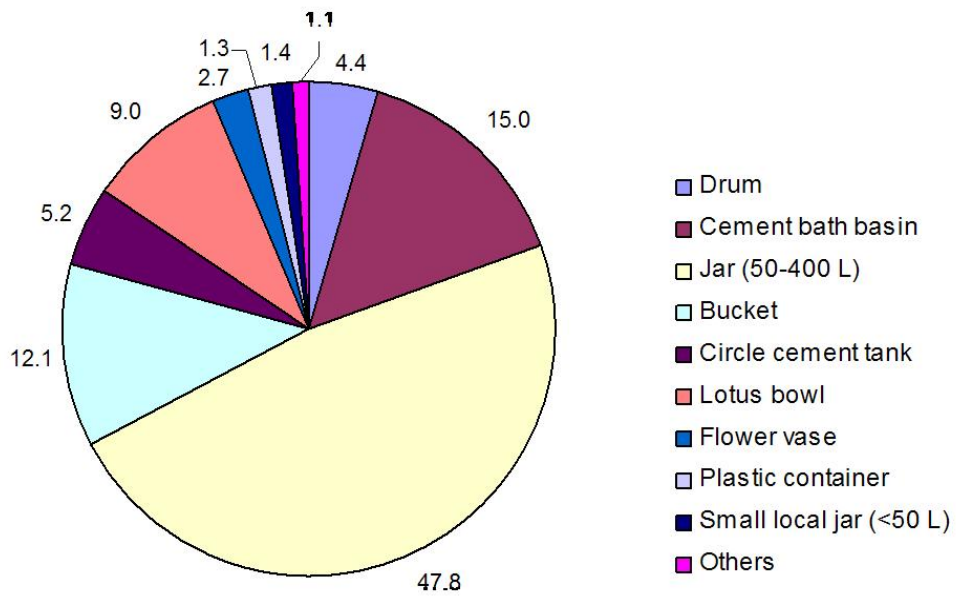
**Figure 44** Percentage contributions to container types observed in cluster no. 8 during the dry season survey.



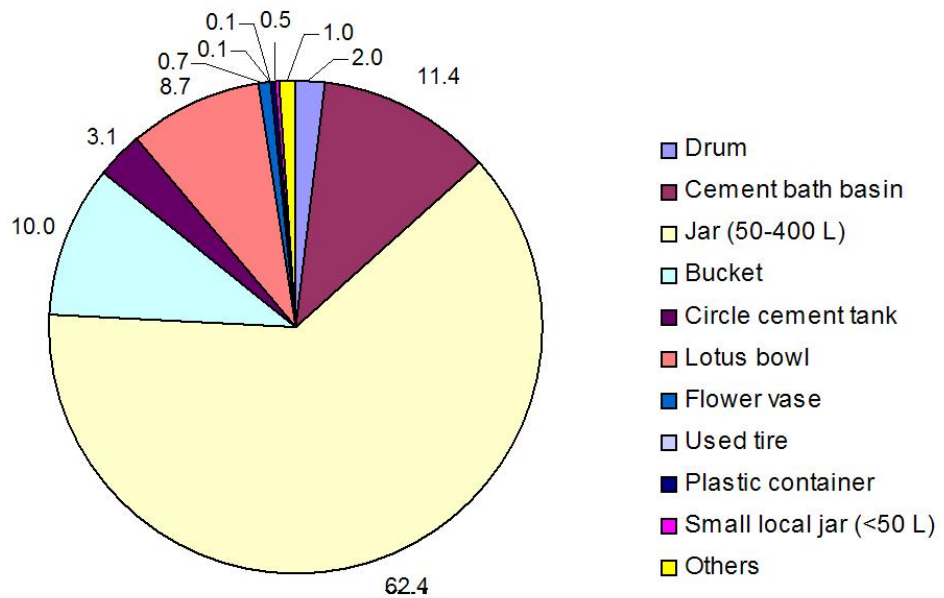
**Figure 45** Percentage contributions to container types observed in cluster no. 9 during the dry season survey.



**Figure 46** Percentage contributions to container types observed in cluster no. 10 during the dry season survey.



**Figure 47** Percentage contributions to container types observed in cluster no. 11 during the dry season survey.



**Figure 48** Percentage contributions to container types observed in cluster no. 12 during the dry season survey.

From the entire 6,836 water-bearing containers observed among the 12 study clusters during the dry season survey, the containers were individually distributed among the 4 ecotopes (RCDENPURA, DENPURA, C, and RC) in amounts of 991, 1,423, 591, and 3,831, respectively.

By using individual chi-square tests, the associations of container characteristics together with their social use in relation to the 4 different ecotopes were made for the dataset of the dry season survey, the results of which are shown in Table 6. Test results revealed that there were significant associations among the 4 different ecotope types and all variables of container characteristics and their social use: type of container ( $P < 0.001$ ), water type ( $P < 0.001$ ), position under vegetation ( $P < 0.001$ ), location ( $P < 0.001$ ), usage in the past 7 days ( $P = 0.027$ ), shade ( $P < 0.001$ ), intervention ( $P < 0.001$ ), and cover ( $P < 0.001$ ). Furthermore, these four individual ecotopes were investigated to determine whether there was an association between the presence of *Aedes* immatures in a habitat and container characteristics, including their social use, the findings of which are shown in Table 7. From a total of 395 *Aedes* immature-positive containers observed among 12 study clusters during the dry season survey, the containers were individually distributed among the 4 ecotopes (RCDENPURA, DENPURA, C, and RC) in amounts of 76, 43, 38, and 238, respectively.

Out of the 4 ecotopes, only the RC ecotope contained the available dataset for the chi-square test, and the results demonstrated a significant association between the presence of mosquito immatures and container type, with  $P < 0.001$ . Regarding water type, only RCDENPURA and DENPURA were eligibly tested, and none of them statistically revealed an association between the presence of *Aedes* immatures and the type of water. Similarly, there was no association between the immature-positive containers and their position under vegetation for the eligible 2 ecotopes, RCDENPURA and RC. The association of the number of immature-positive containers among the individual 4 ecotopes was also investigated with 2 other variables: location and usage in the past 7 days. With regards to location, it was only the RC ecotope that showed a significant association with the number of immature-positive containers, with a P-value of 0.003. In contrast, none of the 4 ecotopes revealed a strong association between usage in the past 7 days and the presence of *Aedes* immatures. Furthermore, all ecotopes except ecotope C were examined for

shade, and among these, only RC demonstrated a strong association between shade and the number of immature-positive containers ( $P=0.001$ ). The last two variables statistically tested were intervention and cover. For intervention, all ecotopes demonstrated a significant association with the number of immature-positive containers, with almost all being  $P<0.001$ . However, only the RCDENPURA, DENPURA, and RC ecotopes showed a significant association between cover and the number of immature-positive containers (all with  $P<0.001$ ).

**Table 6** Number and percent of container characteristics and their social use in relation to 4 different ecotopes surveyed in the dry season.

Container characteristics and their social use	Number of containers (%)				P-value
	RCDENPURA	DENPURA	C	RC	
<b>Type of container</b>					
Drum	34 (3.4)	33 (2.3)	50 (8.5)	268 (7.0)	< 0.001
Cement bath basin	191 (19.3)	138 (9.7)	195 (33.0)	538 (14.0)	
Jar (50-400L)	500 (50.5)	1,075 (75.5)	93 (15.7)	2,025 (52.9)	
Bucket	105 (10.6)	91 (6.4)	107 (18.1)	423 (11.0)	
Circle cement tank	40 (4.0)	25 (1.8)	8 (1.3)	99 (2.6)	
Lotus bowl	69 (7.0)	38 (2.7)	94 (15.9)	312 (8.1)	
Flower vase	30 (3.0)	9 (0.6)	14 (2.4)	48 (1.2)	
Plastic container	4 (0.4)	7 (0.5)	0 (0)	25 (0.7)	
Small local jar	2 (0.2)	1 (0.1)	4 (0.7)	29 (0.8)	
Others	16 (1.6)	6 (0.4)	26 (4.4)	64 (1.7)	
<b>Water type</b>					
Tap water/Well	885 (89.3)	1,005 (70.6)	557 (94.2)	3,258 (85.0)	< 0.001
Rainwater	100 (10.1)	392 (27.6)	26 (4.4)	569 (14.9)	
Canal	6 (0.6)	26 (1.8)	8 (1.4)	4 (0.1)	

**Table 6 (Continued)** Number and percent of container characteristics and their social use in relation to 4 different ecotopes surveyed in the dry season.

Container characteristics and their social use	Number of containers (%)				P-value
	RC	DENPURA	C	RC	
<b>Under vegetation</b>					
Fully	138 (13.9)	133 (9.3)	29 (4.9)	424 (11.1)	< 0.001
Partially	86 (8.7)	98 (6.9)	22 (3.7)	398 (10.4)	
Not	767 (77.4)	1,192 (83.8)	540 (91.4)	3,009 (78.5)	
<b>Location</b>					
Outside	297 (30.0)	535 (37.6)	69 (11.7)	1,456 (38.0)	< 0.001
Inside	694 (70.0)	888 (62.4)	522 (88.3)	2,375 (62.0)	
<b>Usage in the past 7 days</b>					
Yes	698 (70.4)	1,061 (74.6)	414 (70.1)	2,704 (70.6)	0.027
No	293 (29.6)	362 (25.4)	177 (29.9)	1,127 (29.4)	
<b>Shade</b>					
Fully	698 (70.4)	891 (62.6)	520 (88.0)	2,537 (66.2)	< 0.001
Partially	179 (18.1)	343 (24.1)	53 (9.0)	684 (17.9)	
Not	114 (11.5)	189 (13.3)	18 (3.0)	610 (15.9)	
<b>Intervention</b>					
Yes	589 (59.4)	1,062 (74.6)	369 (62.4)	2,551 (66.9)	< 0.001
No	402 (40.6)	361 (25.4)	222 (37.6)	1,280 (33.4)	
<b>Cover</b>					
Preventable	331 (33.4)	753 (52.9)	95 (16.1)	1,670 (43.6)	< 0.001
Partially	80 (8.1)	185 (13.0)	20 (3.4)	217 (5.7)	
Not	580 (58.5)	485 (34.1)	476 (80.5)	1,944 (50.7)	

\*Significantly different ( $P < 0.05$ ) with  $\chi^2$  test for four independent samples.

**Table 7** Number and percent of immature-positive containers in relation to characteristics and their social use among 4 different ecotopes surveyed in the dry season.

Container characteristics and their social use	Number of immature-positive containers (%)			
	RCDENPURA (N=76)	DENPURA (N=43)	C (N=38)	RC (N=238)
<b>Type of container</b>	N/A	N/A	N/A	P < 0.001
Drum	1 (1.3)	0 (0)	1 (2.6)	30 (12.6)
Cement bath basin	21 (27.7)	13 (30.3)	22 (57.9)	60 (25.2)
Jar (50-400L)	36 (47.4)	27 (62.8)	8 (21.1)	77 (32.3)
Bucket	8 (10.5)	1 (2.3)	1 (2.6)	38 (16.0)
Circle cement tank	2 (2.6)	0 (0)	1 (2.6)	5 (2.1)
Lotus bowl	6 (7.9)	0 (0)	1 (2.6)	5 (2.1)
Flower vase	1 (1.3)	0 (0)	3 (7.9)	4 (1.7)
Plastic container	0 (0)	1 (2.3)	0 (0)	5 (2.1)
Small local jar	1 (0.1)	0 (0)	0 (0)	9 (3.8)
Others	0 (0)	1 (2.3)	2 (5.3)	5 (2.1)
<b>Water type</b>	P = 0.133	P = 0.375	N/A	N/A
Tap water/Well	72 (94.7)	34 (79.1)	38 (100.0)	222 (93.3)
Rainwater	3 (4.0)	9 (20.9)	0 (0)	16 (6.7)
Canal	1 (1.3)	0 (0)	0 (0)	0 (0)
<b>Under vegetation</b>	P = 0.238	N/A	N/A	P = 0.063
Fully	9 (11.8)	5 (11.6)	1 (2.6)	28 (11.8)
Partially	3 (4.0)	6 (14.0)	0 (0)	14 (5.9)
Not	64 (84.2)	32 (74.4)	37 (97.4)	196 (82.3)

**Table 7 (Continued)** Number and percent of immature-positive containers in relation to characteristics and their social use among 4 different ecotopes surveyed in the dry season.

Container characteristics and their social use	Number of immature-positive containers (%)			
	RCDENPURA (N=76)	DENPURA (N=43)	C (N=38)	RC (N=238)
<b>Location</b>	P = 0.840	P = 0.311	P = 0.296	P = 0.003
Outside	22 (28.9)	13 (30.2)	2 (5.3)	69 (29.0)
Inside	54 (71.1)	30 (69.8)	36 (94.7)	169 (71.0)
<b>Usage in the past 7 days</b>	P = 0.518	P = 0.161	P = 0.049	P = 0.305
Yes	56 (73.7)	36 (83.7)	32 (84.2)	161 (67.6)
No	20 (26.3)	7 (16.3)	6 (15.8)	77 (32.4)
<b>Shade</b>	P = 0.206	P = 0.096	N/A	P = 0.001
Fully	57 (75.0)	31 (72.1)	35 (92.1)	178 (74.8)
Partially	15 (19.7)	11 (25.6)	1 (2.6)	42 (17.6)
Not	4 (5.3)	1 (2.3)	2 (5.3)	18 (7.6)
<b>Intervention</b>	P < 0.001	P < 0.001	P = 0.001	P < 0.001
Yes	21 (27.6)	19 (44.2)	14 (36.8)	74 (31.1)
No	55 (72.4)	24 (55.8)	24 (63.2)	164 (68.9)
<b>Cover</b>	P < 0.001	P < 0.001	P = 0.061	P < 0.001
Preventable	8 (10.5)	3 (7.0)	2 (5.3)	13 (5.5)
Partially	7 (9.2)	15 (34.9)	3 (7.9)	31 (13.0)
Not	61 (80.3)	25 (58.1)	33 (86.8)	194 (81.5)

\*Significantly different ( $P < 0.05$ ) with  $\chi^2$  test for two independent samples.

N/A = Not available for  $\chi^2$  due to the excess of the expected count being less than 5.

The same 1,200 premises in the study area were re-inspected for an entomological assessment during the dry season. All of the 1,193 houses were found to contain at least one water-holding container and a total of 261 houses were positive for *Aedes* immatures. At the same time, a total of 6,836 containers were observed in the entire area, of which 395 containers were infested and harbored 2,001 *Aedes* immatures. There were 4,160 local inhabitants living in 1,200 houses during the survey. All the entomologic and epidemiologic datasets were further investigated to evaluate dengue transmission risk by applying four entomologic indices: House Index, Container Index, Breteau Index, and pupae per person.

Table 8 compares the entomological indices of *Aedes* vectors with regard to 4 various ecotopes. The House Indices of RCDENPURA, DENPURA, C, and RC were 18.00, 16.00, 15.50, and 27.00, respectively, while the Container Indices of those 4 ecotopes were 7.67, 3.02, 6.43, and 6.21, respectively. The Breteau Indices of the same 4 ecotopes showed 38.00, 21.50, 19.00, and 39.67. Besides these 3 traditional entomological indices, pupae per person (the more effective pupal-demographic index) was also applied to determine dengue transmission risk. Results of this index for all 4 ecotopes were 0.34, 0.24, 0.14, and 0.39, respectively.

**Table 8** Larval and pupal indices of *Aedes* mosquitoes with respect to 4 different ecotopes surveyed in the dry season.

<b>Larval and Pupal Indices</b>	<b>Residential mixed with commercial and DENPURA</b>	<b>DENPURA</b>	<b>Commercial area</b>	<b>Residential mixed with commercial area</b>
House Index (HI)	18.00	16.00	15.50	27.00
Container Index (CI)	7.67	3.02	6.43	6.21
Breteau Index (BI)	38.00	21.50	19.00	39.67
Pupae per Person (PI)	0.34	0.24	0.14	0.39

### **4.3.2 The application of mosquito blood meal analysis to assess dengue herd immunity in human populations**

The *Culex* mosquito blood meals collected during the wet and dry seasons were used to examine human herd immunity against all serotypes of dengue viruses among 4 different ecotopes of urban and semi-urban settings in the study areas. The seasonality seroprevalence of anti-dengue IgM and IgG antibodies was also determined among the 12 studied clusters.

#### **4.3.2.1 Dengue herd immunity in the wet season**

From 715 blood-fed *Culex* mosquitoes collected indoors from the 1200 households in the wet season and serologically examined for the presence of human IgG antibody by using ELISA, 390 blood meals (54.5%) tested positive for human IgG (Table 9). Among all *Culex* species identified, *Cx. quinquefasciatus* was commonly found (88.5%), while the remainder accounted for only 11.5%. Human IgG seropositivity rates varied from 0% to 100% among species. The highest human IgG seropositivity rate was found in *Cx. Sitiens* (100%), followed by *Cx. tritaeniorhynchus* (80%), *Cx. gelidus* (61.3%), *Cx. quinquefasciatus* (54.8%), *Cx. hutchinsoni* (46.2%), and *Cx. vishnui* (44.0%). The lowest human IgG seropositivity rate (0%) was discovered in *Cx. brevialpis* and *Cx. cinetellus*. There was no significant difference among human IgG-seropositive *Culex* samples during this season ( $\chi^2=8.860$ ,  $df=8$ ,  $P=0.354$ ).

**Table 9** Number and percent of blood-fed *Culex* mosquitoes collected in the wet season, determined by human IgG ELISA.

Species	N (%)	Human IgG	
		Positive (%)	Negative (%)
<i>Cx. quinquefasciatus</i>	633 (88.5)	347 (54.8)	286 (45.2)
<i>Cx. gelidus</i>	31 (4.4)	19 (61.3)	12 (38.7)
<i>Cx. vishuni</i>	25 (3.5)	11 (44.0)	14 (56.0)
<i>Cx. hutchisoni</i>	13 (1.8)	6 (46.2)	7 (53.8)
<i>Cx. tritaeniorhynchus</i>	5 (0.7)	4 (80.0)	1 (20.0)
<i>Cx. whitmani</i>	4 (0.6)	1 (25.0)	3 (75.0)
<i>Cx. sitiens</i>	2 (0.3)	2 (100)	0 (0)
<i>Cx. brevipalpis</i>	1 (0.1)	0 (0)	1 (100)
<i>Cx. cinetellus</i>	1 (0.1)	0 (0)	1 (100)
Total	715 (100)	390 (54.5)	325 (45.5)

To analyze the distribution of human IgG-seropositive *Culex* samples among all 12 study clusters, all 6-pair clusters with high and low degrees of dengue transmission were used. Human IgG-seropositivity of *Culex* blood meals individually ranged from 28.7% to 90.0%, but each paired cluster did not differ significantly (Table 10). On the other hand, 77.5% of the samples obtained from 6 semi-urban clusters were positive—this being nearly 2-fold higher than that derived from all urban clusters (41.6%). This difference in human IgG-seropositivity between semi-urban and urban clusters was remarkably significant ( $\chi^2= 85.930$ ,  $df=1$ ,  $P<0.001$ ).

For an examination of dengue herd immunity in human populations, the 390 human IgG-seropositive blood meals were further investigated by the immunochromatographic test. Overall, seropositive test results revealed percentages of 17.4% and 10.3% for DENV IgM and IgG, respectively (Table 11). Among all 12 study clusters, the DENPURA with a high degree of dengue transmission showed the highest DENV IgM seropositivity rate (44.1%), whereas the highest measures for

DENV IgG seropositivity were observed in the RCDENPURA with a low dengue transmission degree (20.0%), DENPURA with a high degree of dengue transmission (20.6%), and RC with a high degree of dengue transmission (20.8%). No significant difference in seropositivity for either DENV IgM or DENV IgG was observed between urban and semi-urban areas. In contrast, both DENV IgM and IgG seropositivity rates were significantly higher in a high dengue transmission area than in a low dengue transmission area, with P-values of 0.006 and 0.003, respectively. These DENV IgM and IgG seropositivity rates were then distinctly compared among the same ecotopes to see the differences between the two degrees of dengue transmission and the diverse ecotopes. The DENPURA with a high degree of dengue transmission (cluster 3) showed a higher DENV IgM-seropositivity rate (44.1%) than that of the DENPURA (10.3%) with a low dengue transmission degree (cluster 4), with a significant difference ( $P=0.003$ ). Similarly, this significant difference ( $P=0.013$ ) was observed for the DENV IgG-seropositivity rates of the two clusters. Additionally, the ecotope of residential mixed with a commercial area (RC) with a high dengue transmission degree (cluster 9) and with a low dengue transmission degree (cluster 10) differed significantly in the DENV IgG seropositivity rate, with a P-value of 0.038.

Table 12 shows the ecotope characteristics as risk factors for DENV IgM- and IgG-seropositive samples in relation to human population dengue herd immunity. Urbanization was not statistically associated with DENV IgM seropositivity. Conversely, there was a significant association ( $OR=2.117$ ) between the degree of dengue transmission and DENV IgM seroprevalence, with 22.3% and 12.0% of DENV IgM seropositivity in high and low dengue transmission areas, respectively. With respect to the ecotopes lying between urban and semi-urban areas, the seropositive rates varied from 5% to 29% among all 4 ecotopes. Interestingly, three common ecotopes (DENPURA, RCDENPURA, and RC) indicated increased risks ( $OR>3.0$ ) of DENV IgM seropositivity, compared to the commercial ecotope. However, only DENPURA had a highly significant OR, 7.0.

Similar to DENV IgM seroprevalence, urbanization also was not associated with DENV IgG seropositivity. However, a significant association ( $OR=1.984$ ) was observed for the degree of dengue transmission, with 13.1% and 7.1% of DENV IgG seropositivity being shown in high and low dengue transmission

areas, respectively. In contrast to DENV IgM seropositivity-associated ecotopes, these three common ecotopes did not exhibit association with DENV IgG seropositivity.

By combining data on DENV IgM and DENV IgG seropositivity, higher seropositive rates in all ecotopes fell into either the urbanization or the degree of dengue transmission categories, except for the commercial ecotope. Unlike urbanization, the degree of dengue transmission got significantly associated (OR=2.029) with a DENV IgM/IgG seroprevalence of 24.8% and 13.6% in high and low dengue transmission areas. With respect to the ecotopes lying between urban and semi-urban areas, higher seropositive rates of 30.2%, 20.0%, and 18.5% were obtained from three common ecotopes: DENPURA, RCDENPURA, and RC, respectively. As a result, they all showed higher odds ratios (OR=7.6, 4.4, and 4.0) for DENV IgM/IgG seropositivity. Again, the ecotope of DENPURA still had a highly significant OR, 7.6.

**Table 10** Number and percent of human IgG-seropositive blood-fed *Culex* mosquitoes collected in the wet season in relation to study clusters.

Cluster No.	Type of ecotope	Degree of dengue transmission	N (%)	Human IgG	
				Positive (%)	Negative (%)
1	Residential mixed with commercial area and DENPURA	High	12 (16.8)	50 (41.7)	70 (58.3)
2	Residential mixed with commercial area and DENPURA	Low	82 (11.5)	40 (48.8)	42 (51.2)
3	DENPURA	High	82 (11.5)	34 (41.5)	48 (58.5)
4	DENPURA	Low	101(14.1)	29 (28.7)	72 (71.3)
5	Commercial area	High	33 (4.6)	14 (42.4)	19 (57.6)
6	Commercial area	Low	39 (5.5)	23 (59.0)	16 (41.0)
7	Residential mixed with commercial area	High	32 (4.5)	25 (78.1)	7 (21.9)
8	Residential mixed with commercial area	Low	10 (1.4)	9 (90.0)	1 (10.0)
9	Residential mixed with commercial area	High	76 (10.6)	59 (77.6)	17 (22.4)
10	Residential mixed with commercial area	Low	68 (9.5)	49 (72.1)	19 (27.9)
11	Residential mixed with commercial area	High	31 (4.3)	24 (77.4)	7 (22.6)
12	Residential mixed with commercial area	Low	41 (5.7)	34 (82.9)	7 (17.1)
	Total		715 (100)	390 (54.5)	325 (45.5)

**Table 11** DENV IgM and IgG seropositivity of human IgG-seropositive *Culex* samples collected in the wet season in relation to study clusters.

Cluster No.	Type of ecotope	Degree of dengue transmission	N (%)	DENV IgM		DENV IgG	
				Positive (%)	Negative (%)	Positive (%)	Negative (%)
1	Residential mixed with commercial area and DENPURA	High	50 (12.8)	8 (16.0)	42 (84.0)	4 (8.0)	46 (92.0)
2	Residential mixed with commercial area and DENPURA	Low	40 (10.3)	7 (17.5)	33 (82.5)	8 (20.0)	32 (80.0)
3	DENPURA	High	34 (8.7)	<sup>a</sup> 15 (44.1)	<sup>a</sup> 19 (55.9)	<sup>b</sup> 7 (20.6)	<sup>b</sup> 27 (79.4)
4	DENPURA	Low	29 (7.4)	<sup>a</sup> 3 (10.3)	<sup>a</sup> 26 (89.7)	<sup>b</sup> 0 (0)	<sup>b</sup> 29 (100)
5	Commercial area	High	14 (3.6)	1 (7.1)	13 (92.9)	1 (7.1)	13 (92.9)
6	Commercial area	Low	23 (5.9)	1 (4.3)	22 (95.7)	1 (4.3)	22 (95.7)
7	Residential mixed with commercial area	High	25 (6.4)	3 (12.0)	22 (88.0)	2 (8.0)	23 (92.0)
8	Residential mixed with commercial area	Low	9 (2.3)	0 (0)	9 (100)	0 (0)	9 (100)
9	Residential mixed with commercial area	High	59 (15.1)	16 (27.1)	43 (72.9)	<sup>c</sup> 8 (13.6)	<sup>c</sup> 51 (86.4)
10	Residential mixed with commercial area	Low	49 (12.6)	6 (12.2)	43 (87.8)	<sup>c</sup> 1 (2.0)	<sup>c</sup> 48 (98.0)
11	Residential mixed with commercial area	High	24(6.2)	3 (12.5)	21 (87.5)	5 (20.8)	19 (79.2)
12	Residential mixed with commercial area	Low	34 (8.7)	5 (14.7)	29 (85.3)	3 (8.8)	31 (91.2)
Total			390 (100)	68 (17.4)	322 (82.6)	40 (10.3)	350 (89.7)

Significant for two-independent samples: <sup>a</sup> $\chi^2=8.747$ ,  $P=0.003$ ; <sup>b</sup>Fisher's Exact test,  $P=0.013$ ; <sup>c</sup>Fisher's Exact test,  $P=0.038$ .



#### 4.3.2.2 Dengue herd immunity in the dry season

Of the 760 blood-fed *Culex* mosquitoes collected indoors during the dry season from the same 1200 households earlier surveyed during the wet season and serologically examined for the presence of human IgG antibody by using ELISA, 253 of the blood meals (33.3%) tested positive for human IgG (Table 13). Among all *Culex* species examined, *Cx. quinquefasciatus* (97.4%) was commonly observed, while the remainder was only 2.6%. Human IgG seropositivity rates varied from 0% to 36.4% among species. The highest human IgG seropositivity rate was found in *Cx. sitiens* (36.4%), followed by *Cx. quinquefasciatus* (33.5%), and *Cx. vishnui* (14.3%). The lowest human IgG seropositivity rate (0%) was seen in *Cx. gelidus* and *Cx. fuscocephala*. There was no significant difference among human IgG-seropositive *Culex* samples during this season ( $\chi^2=2.200$ ,  $df=4$ ,  $P=0.699$ ).

**Table 13** Number and percent of blood-fed *Culex* mosquitoes collected in the dry season, determined by human IgG ELISA.

Species	N (%)	Human IgG	
		Positive (%)	Negative (%)
<i>Cx. quinquefasciatus</i>	740 (97.4)	248 (33.5)	492 (66.5)
<i>Cx. sitiens</i>	11 (1.5)	4 (36.4)	7 (63.6)
<i>Cx. vishuni</i>	7 (0.9)	1 (14.3)	6 (85.7)
<i>Cx. gelidus</i>	1 (0.1)	0 (0)	1 (100)
<i>Cx. fuscocephala</i>	1 (0.1)	0 (0)	1 (100)
Total	760 (100)	253 (33.3)	507 (66.7)

To analyze the distribution of human IgG-seropositive *Culex* samples among all 12 study clusters, all 6-pair clusters with high and low degrees of dengue transmission were used. Human IgG-seropositivity of *Culex* blood meals individually ranged from 20.3% to 75.0%. However, only clusters No. 11 and No. 12 demonstrated statistically significant differences ( $\chi^2= 9.804$ ,  $df=1$ ,  $P=0.002$ ), as shown in Table 14. In light of urbanization, the human IgG-seropositive rate was 41.7% in semi-urban

areas and only 28.7% in urban areas. This difference in human IgG-seropositivity between semi-urban and urban clusters was remarkably significant ( $\chi^2= 13.126$ ,  $df=1$ ,  $P<0.001$ ).

The presence of anti-dengue IgM and IgG antibodies in 253 human IgG-seropositive samples was further investigated by using the immunochromatographic test. Of 253 specimens tested, a total of 96 (37.9%) and 90 (35.6%) samples were serologically positive for DENV IgM and IgG, respectively (Table 15). Among all 12 study clusters, the DENPURA with a high degree of dengue transmission showed the highest seropositivity rates for both DENV IgM (62.5%) and DENV IgG (56.2%). No significant difference in seropositivity for either DENV IgM or DENV IgG was observed between urban and semi-urban areas. Unlike in the urbanization category, both DENV IgM and IgG seropositivity rates were significantly higher in the high dengue transmission area than in the low dengue transmission area, with P-values of 0.007 and 0.050, respectively. These DENV IgM and IgG seropositivity rates then were distinctly compared among the same ecotopes to see the differences between the degrees of dengue transmission and the diverse ecotopes. The DENPURA with a high degree of dengue transmission (cluster 3) showed a higher DENV IgM-seropositivity rate (62.5%) than that of the DENPURA (32.3%) with a low degree of dengue transmission (cluster 4), with a significant difference ( $P=0.047$ ). Also, the same two commercial ecotopes with a high degree of dengue transmission (cluster 5) and a low dengue transmission (cluster 6) differed significantly in the DENV IgG seropositivity rate ( $P=0.037$ ).

Table 16 shows ecotope characteristics as risk factors for DENV IgM- and IgG-seropositive samples in relation to human population dengue herd immunity. Urbanization was not statistically associated with DENV IgM seropositivity. Conversely, there was a significant association ( $OR=2.113$ ) between the degree of dengue transmission and DENV IgM seroprevalence, with 45.0% and 27.9% DENV IgM seropositivity in high and low dengue transmission areas, respectively. With respect to all 4 ecotopes lying between urban and semi-urban areas, the seropositive rates ranged from 5% to 29% among them. Compared to the commercial ecotope, however, three common ecotopes (DENPURA, RCDENPURA, and RC) demonstrated no risk of DENV IgM seropositivity.

Like DENV IgM seroprevalence, urbanization also was not associated with DENV IgG seropositivity. Still, a significant association (OR=2.259) was observed for the degree of dengue transmission, with 43.0% and 25.0% of DENV IgG seropositivity being shown in high and low dengue transmission areas, respectively. In contrast to DENV IgM seropositivity-associated ecotopes, the ecotope of RCDENPURA exhibited strong association with DENV IgG seropositivity (OR=2.667).

Since the combined use of DENV IgM and DENV IgG seropositivity was applied, the seropositive rates among ecotope characteristics became higher except for in the commercial and DENPURA ecotopes. In contrast to the urbanization category, the degree of dengue transmission got significantly associated (OR=1.806) with a DENV IgM/IgG seroprevalence of 45.6% and 31.7% in high and low dengue transmission areas. As for all 4 ecotopes lying between urban and semi-urban areas, higher seropositive rates of 46.9% and 36.9% were obtained from RCDENPURA and RC, respectively. In this case, all of 4 ecotopes revealed no association with DENV IgM/IgG seropositivity.

**Table 14** Number and percent of human IgG-seropositive blood-fed *Culex* mosquitoes collected in the dry season in relation to study clusters.

Cluster No.	Type of ecotope	Degree of dengue transmission	N (%)	Human IgG	
				Positive (%)	Negative (%)
1	Residential mixed with commercial area and DENPURA	High	106 (14.0)	37 (34.9)	69 (65.1)
2	Residential mixed with commercial area and DENPURA	Low	61 (8.0)	27 (44.3)	34 (55.7)
3	DENPURA	High	79 (10.4)	16 (20.3)	63 (79.7)
4	DENPURA	Low	153 (20.1)	31 (20.3)	122 (79.7)
5	Commercial area	High	54 (7.1)	15 (27.8)	39 (72.2)
6	Commercial area	Low	41 (5.4)	16 (39.0)	25 (61.0)
7	Residential mixed with commercial area	High	109 (14.3)	32 (29.4)	77 (70.6)
8	Residential mixed with commercial area	Low	30 (4.0)	9 (30.0)	21 (70.0)
9	Residential mixed with commercial area	High	48 (6.3)	36 (75.0)	12 (25.0)
10	Residential mixed with commercial area	Low	15 (2.0)	11 (73.3)	4 (26.7)
11	Residential mixed with commercial area	High	50 (6.6)	13 (26.0)	37 (74.0)
12	Residential mixed with commercial area	Low	14 (1.8)	10 (71.4)	4 (28.6)
Total			760 (100)	253 (33.3)	507 (66.7)

**Table 15** DENV IgM and IgG seropositivity of human IgG-seropositive *Culex* samples collected in the dry season in relation to study clusters.

Cluster No.	Type of ecotope	Degree of dengue transmission	N (%)	DENV IgM		DENV IgG	
				Positive (%)	Negative (%)	Positive (%)	Negative (%)
1	Residential mixed with commercial area and	High	37 (14.6)	18 (48.6)	19 (51.4)	17 (45.9)	20 (54.1)
2	Residential mixed with commercial area and	Low	27 (10.7)	9 (33.3)	18 (66.7)	11 (40.7)	16 (59.3)
3	DENPURA	High	16 (6.3)	<sup>a</sup> 10 (62.5)	<sup>a</sup> 6 (37.5)	9 (56.2)	7 (43.8)
4	DENPURA	Low	31 (12.3)	<sup>a</sup> 10 (32.3)	<sup>a</sup> 21 (67.7)	9 (29.0)	22 (71.0)
5	Commercial area	High	15 (5.9)	6 (40.0)	9 (60.0)	<sup>b</sup> 6 (40.0)	<sup>b</sup> 9 (60.0)
6	Commercial area	Low	16 (6.3)	4 (25.0)	12 (75.0)	<sup>b</sup> 1 (6.2)	<sup>b</sup> 15 (93.8)
7	Residential mixed with commercial area	High	32 (12.6)	16 (50.0)	16 (50.0)	15 (46.9)	17 (53.1)
8	Residential mixed with commercial area	Low	9 (3.6)	1 (11.1)	8 (88.9)	2 (22.2)	7 (77.8)
9	Residential mixed with commercial area	High	36 (14.2)	12 (33.3)	24 (66.7)	12 (33.3)	24 (66.7)
10	Residential mixed with commercial area	Low	11 (4.4)	2 (18.2)	9 (81.8)	2 (18.2)	9 (81.8)
11	Residential mixed with commercial area	High	13 (5.1)	5 (38.5)	8 (61.5)	5 (38.5)	8 (61.5)
12	Residential mixed with commercial area	Low	10 (4.0)	3 (30.0)	7 (70.0)	1 (10.0)	9 (90.0)
Total			253 (100)	96 (37.9)	157 (62.1)	90 (35.6)	163 (64.4)

Significant for two-independent samples: <sup>a</sup> $\chi^2=3.948$ ,  $P=0.047$ ; <sup>b</sup>Fisher's Exact test,  $P=0.037$ .

**Table 16** Risk factors for DENV IgM/IgG-seropositive blood-meal *Culex* samples in the dry season in relation to human population herd immunity.

Characteristics	N	DENV IgM		DENV IgG		DENV IgM/IgG			
		Positive (%)	OR	P-value	Positive (%)	OR	P-value		
<b>Urbanization</b>									
Semi-urban	111	39 (35.1)	1		37 (33.3)		41 (36.9)	1	
Urban	142	57 (40.1)	1.238	0.416	53 (37.3)	1.191	60 (42.3)	1.249	0.392
<b>Degree of dengue transmission</b>									
Low	104	29 (27.9)	1		26 (25.0)	1	33 (31.7)	1	
High	149	67 (45.0)	2.113	0.006	64 (43.0)	2.259	68 (45.6)	1.806	0.026
<b>Ecotopes</b>									
<b>Urban</b>									
Commercial area	31	10 (32.3)	1		7 (22.6)	1	10 (32.3)	1	
DENPURA	47	20 (42.6)	1.556	0.360	18 (38.3)	2.128	20 (42.6)	1.556	0.360
Residential mixed with Commercial area and DENPURA	90	27 (42.2)	1.532	0.352	28 (43.8)	2.667	30 (46.9)		0.176
<b>Semi-urban</b>									
Residential mixed with Commercial area	111	39 (35.1)	1.138	0.766	37 (33.3)	1.714	41 (36.9)		0.631

#### **4.3.2.3 Seasonal and spatial analysis of anti-dengue human herd immunity and its relation to reported dengue cases**

The seasonal seroprevalence of anti-dengue IgM and IgG antibodies affecting human herd immunity and their spatial distribution were established among the 12 studied clusters. Finally, this seasonal seroprevalence of antibodies, which was derived from the blood meal analysis in both seasons, was related to the actual dengue cases reported during the same period of time. Overall, the seroprevalence rates of both DENV IgM and IgG antibodies in the dry season were significantly higher than those in the wet season ( $\chi^2=33.970$ ,  $df=1$ ,  $P<0.001$ ;  $\chi^2=60.975$ ,  $df=1$ ,  $P<0.001$ ). The DENV IgM seroprevalence of individual clusters was compared between two different seasons (Figure 47). Out of all 12 clusters, clusters no.1, 4, and 7 demonstrated a higher DENV IgM seropositivity rate in the dry season than those of the wet season, with a significant difference ( $P=0.001$ ,  $P=0.040$  and  $P=0.003$ ). The DENV IgG seroprevalence derived from the same individual clusters was also examined in a similar way, with the results shown in Figure 49. In this case, a total of 5 out of 12 clusters revealed a significant association between the DENV seropositivity rate and the season. These included clusters no. 1, 3, 4, 7, and 9, with P-values of  $<0.001$ , 0.012, 0.002, 0.001, and 0.002, respectively.

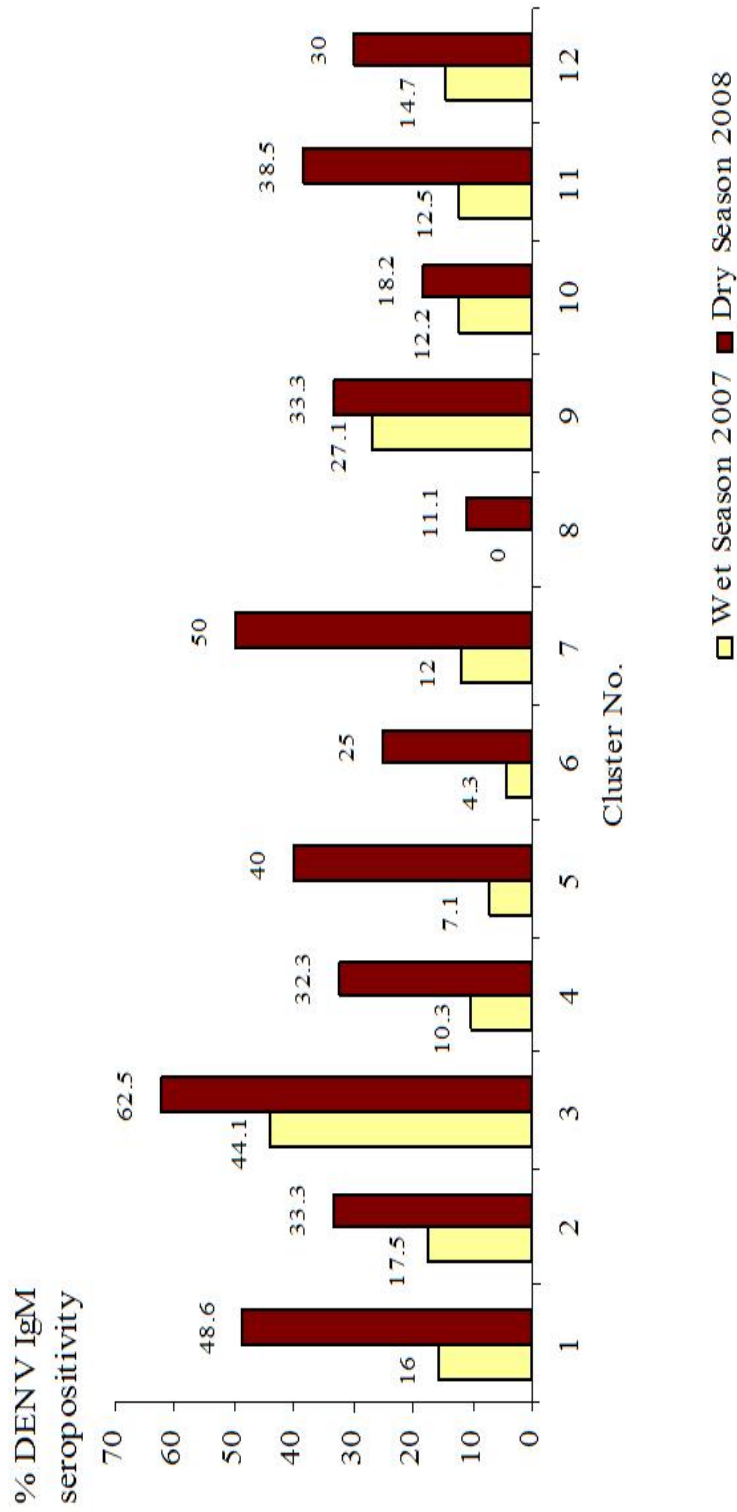
Table 17 shows an association between DENV IgM/IgG seroprevalence in relation to ecotope characteristics and different seasons. The seasonality pattern of DENV IgM and IgG seropositive rates were statistically determined in light of ecotope characteristics. For DENV IgM, a significant difference in seropositivity, both in semi-urban or urban areas, was observed between wet and dry seasons with P-values of  $<0.001$  and  $<0.001$ , respectively. In this regard, two different degrees of dengue transmission areas both exhibited a higher DENV IgG seroprevalence during the dry season than that derived in the wet season ( $P=0.001$ ;  $P<0.001$ ). With respect to all 4 ecotopes lying between urban and semi-urban areas, a significant association between DENV IgM seropositivity and season was observed in 2 ecotopes of commercial area and RCDENPURA with P-values of 0.004 and  $<0.001$ , respectively.

Regarding DENV IgG, the seroprevalence of semi-urban and urban areas showed a statistical difference between the wet and dry seasons, with P-values of  $<0.001$  and  $<0.001$ . This significant difference existed as well for two areas where one

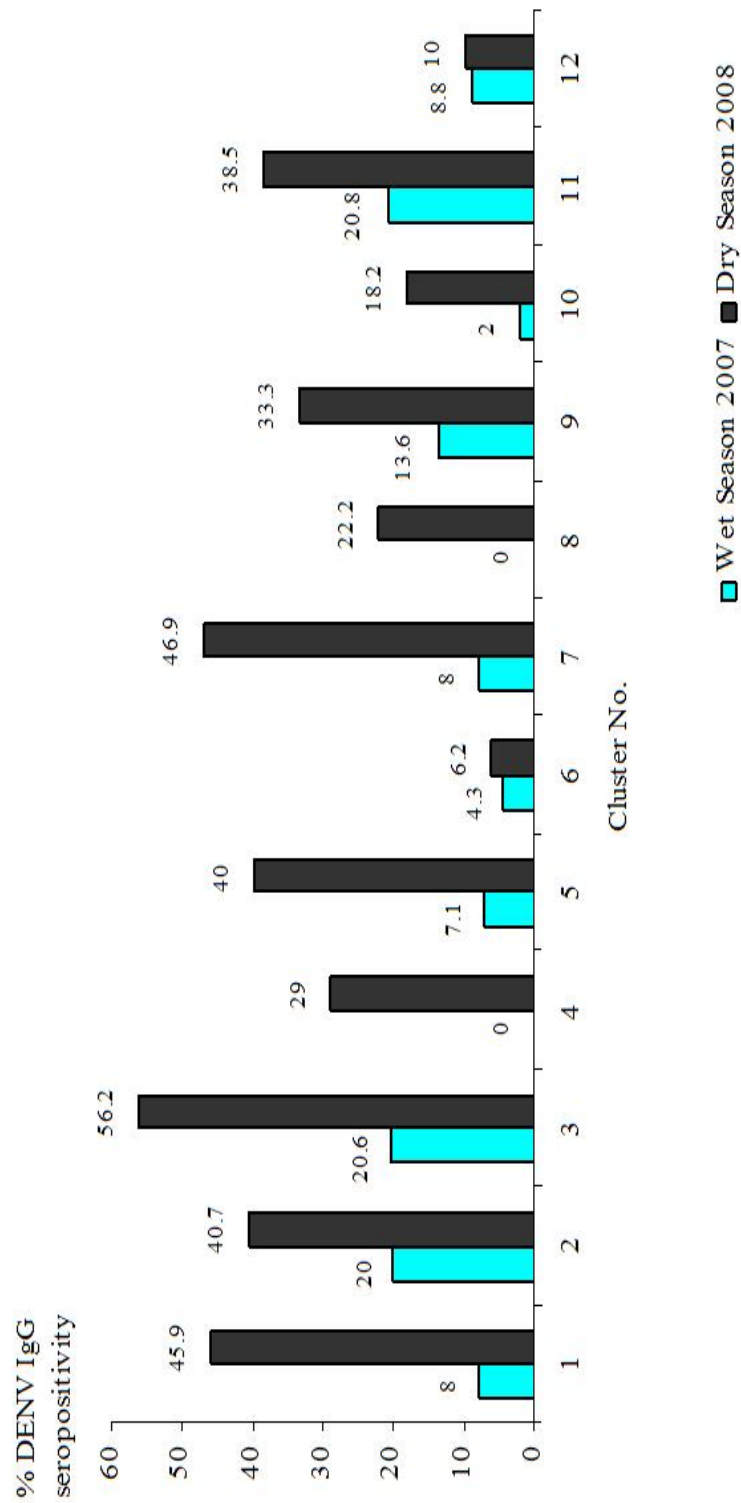
had a high and one had a low degree of dengue transmission ( $P < 0.001$  and  $P < 0.001$ ). Concerning the 4 different ecotopes lying between urban and semi-urban settings, a significant association between DENV IgG seroprevalence and the season was observed in all ecotopes of Commercial area, DENPURA, RCDENPURA, and RC, with P-values of 0.037, 0.001,  $< 0.001$ , and  $< 0.001$ , respectively.

In combining DENV IgM and IgG seropositivity, there were statistically significant differences in the seroprevalence of urban ( $P < 0.001$ ) and semi-urban ( $P < 0.001$ ) areas between the wet and dry seasons. These significant differences were also observed in the two settings with high and low degrees of dengue transmission ( $P < 0.001$  and  $P < 0.001$ ). Similar to DENV IgM alone, a strong association between DENV IgM/IgG seroprevalence and season was found in all types of ecotopes with the same P-values, except for the ecotope of DENPURA.

Eventually, anti-dengue seroprevalence findings obtained from blood meal analyses were related cluster by cluster to the numbers of actual dengue cases reported in each cluster at the same period of time. In comparing a pair of identical ecotoped clusters with high and low degrees of dengue transmission, almost all of the clusters showed a relation between the DENV seropositivity rate and reported number of dengue cases (Table 18). The paired clusters of DENPURA with a high (cluster 3) and low degree (cluster 4) of dengue transmission that differed significantly in DENV IgM seroprevalence correlated very well with the numbers of dengue cases reported during the same period of time during the wet season of 2007 and the dry season of 2008. The statistically significant differences in the DENV IgG seropositivity rates of two paired clusters of DENPURA (clusters 3 and 4) and RC (cluster 9 and 10) in the wet season of 2007 demonstrated a strong correlation of DENV IgG-positive numbers with dengue case numbers. For the dry season in 2008, a pair of commercial clusters with a significant difference in DENV IgG seropositivity showed a powerful correlation between the number of positive DENV IgM and the number of dengue cases reported from the same clusters.



**Figure 49** Comparison of DENV IgM seropositivity of mosquito blood meal samples among individual 12 study clusters, between the wet season in 2007 and the dry season in 2008.



**Figure 50** Comparison of DENV IgG seropositivity of mosquito blood meal samples among individual 12 study clusters between the wet season in 2007 and the dry season in 2008.

**Table 17** DENV IgM/IgG seroprevalence in relation to human herd immunity among ecotopes between the dry and wet seasons.

Characteristics	N	Anti-dengue seropositivity (%)								
		DENV IgM			DENV IgG					
		Wet	Dry	P-value	Wet	Dry	P-value			
<b>Urbanization</b>										
Semi-urban	311	33 (16.5)	39 (35.1)	< 0.001	19 (9.5)	37 (33.3)	< 0.001	37 (18.5)	41 (36.9)	< 0.001
Urban	332	35 (18.4)	57 (40.1)	< 0.001	21 (11.1)	53 (37.3)	< 0.001	39 (20.5)	60 (42.3)	< 0.001
<b>Degree of dengue transmission</b>										
Low	288	22 (12.0)	29 (27.9)	0.001	13 (7.1)	26 (25.0)	< 0.001	25 (13.6)	33 (31.7)	< 0.001
High	355	46 (22.3)	67 (45.5)	< 0.001	27 (13.1)	64 (43.0)	< 0.001	51 (24.8)	68 (45.6)	< 0.001
<b>Ecotopes</b>										
<b>Urban</b>										
Commercial area	68	2 (5.4)	10 (32.3)	0.004	2 (5.4)	7 (22.6)	0.037	2 (5.4)	10 (32.3)	0.004
DENPURA	110	18 (28.6)	20 (42.6)	0.127	7 (11.1)	18 (38.3)	0.001	19 (30.2)	20 (42.6)	0.179
Residential mixed with Commercial area and DENPURA	114	15 (16.7)	27 (42.2)	< 0.001	12 (13.3)	28 (43.8)	< 0.001	18 (20.0)	30 (46.9)	< 0.001
<b>Semi-urban</b>										
Residential mixed with Commercial area	311	33 (16.5)	39 (35.1)	< 0.001	19 (9.5)	37 (33.3)	< 0.001	37 (18.5)	41 (36.9)	< 0.001

**Table 18** Association of annual reported numbers of dengue and dengue haemorrhagic fever cases in studied communities from 2003-2008 and DENV IgM/IgG seroprevalence in relation to human herd immunity.

Cluster No.	Ecotope types	Degree of dengue transmission	Annual reported number of dengue cases								Seropositivity of <i>Culex</i> blood meal		
			Total	2003	2004	2005	2006	2007	2008	2007	2008	DENV IgM	DENV IgG
1	Residential mixed Commercial area & DENPURA	High	32	4	8	10	3	5	2	8 (16.0)	18 (48.6)	4 (8.0)	17 (45.9)
2	Residential mixed Commercial area & DENPURA	Low	17	1	2	3	2	7	2	7 (17.5)	9 (33.3)	8 (20.0)	11 (40.7)
3	DENPURA	High	53	2	13	5	11	11	11	<sup>a</sup> 15 (44.1) <sup>d</sup> 10 (62.5)	<sup>b</sup> 7 (20.6)	9 (56.2)	
4	DENPURA	Low	16	7	3	0	1	2	3	<sup>a</sup> 3 (10.3) <sup>d</sup> 10 (32.3)	<sup>b</sup> 0 (0)	9 (29.0)	
5	Commercial area	High	57	2	10	8	11	7	19	1 (7.1)	6 (40.0)	1 (7.1)	<sup>e</sup> 6 (40.0)
6	Commercial area	Low	24	6	1	4	0	5	8	1 (4.3)	4 (25.0)	1 (4.3)	<sup>e</sup> 1 (6.2)
7	Residential mixed with commercial area	High	35	6	8	7	6	1	7	3 (12.0)	16 (50.0)	2 (8.0)	15 (46.9)
8	Residential mixed with commercial area	Low	13	2	0	1	0	5	5	0 (0)	1 (11.1)	0 (0)	2 (22.2)
9	Residential mixed with commercial area	High	31	10	8	5	3	3	2	16 (27.1)	12 (33.3)	<sup>e</sup> 8 (13.6)	12 (33.3)
10	Residential mixed with commercial area	Low	5	0	2	0	3	0	0	6 (12.2)	2 (18.2)	<sup>e</sup> 1 (2.0)	2 (18.2)
11	Residential mixed with commercial area	High	34	5	4	4	7	8	6	3 (12.5)	5 (38.5)	5 (20.8)	5 (38.5)
12	Residential mixed with commercial area	Low	12	1	2	0	3	0	6	5 (14.7)	3 (30.0)	3 (8.8)	1 (10.0)

## 4.4 Social Science Research

### 4.4.1 Examination of socio-demographic parameters underlying the dengue transmission

A total of 1,200 respondents of all individual studied households in high and low dengue transmission areas were interviewed to examine the general characteristics and previous history of dengue infection of household members, housing, and basic services, knowledge about dengue, and vector related knowledge and practices.

The background characteristics of 1,200 heads of household and 1,200 respondents in high and low transmission areas were analyzed by proportion testing, and the results are presented in Table 19. The background features taken into account encompassed gender, age, religion, years of schooling, highest school degree attained, and occupation. Research findings indicated that no significant differences were found in the percentage of individual characteristics of household leaders and respondents when a comparison was made between high and low dengue transmission clusters.

The general characteristics of 4,166 household members dwelling in 1,200 studied households located in high and low transmission areas were also used to describe significant differences, if any, between the two types of households (Table 20). Of all characteristics, only the previous history of dengue infection demonstrated a higher significant number for persons living in a high transmission area than those dwelling in a low transmission locality.

**Table 19** Background characteristics of heads of household and respondents living in high and low dengue transmission areas.

Characteristics	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission	Low dengue transmission	
		(N=600)	(N=600)	
<b>Head of household (N=1,200)</b>				
<i>Gender</i>				
Male	718 (59.8)	368 (61.3)	350 (58.3)	0.289
Female	482 (40.2)	232 (38.7)	250 (41.7)	
<i>Age</i>				
18-34	218 (18.2)	104 (17.3)	114 (19.0)	0.454
≥ 35	982 (81.8)	496 (82.7)	486 (81.0)	

**Table 19 (Continued)** Background characteristics of heads of household and respondents living in high and low dengue transmission areas.

Characteristics	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission	Low dengue transmission	
		(N=600)	(N=600)	
<i>Religion</i>				
Buddhist	1,181 (98.4)	587 (97.8)	594 (99.0)	0.105
Muslim/Christian	19 (1.6)	13 (2.2)	6 (1.0)	
<i>Years of schooling</i>				
0-7	655 (54.6)	326 (54.3)	329 (54.8)	0.892
8-14	355 (29.6)	176 (29.4)	179 (29.8)	
15-21	190 (15.8)	98 (16.3)	92 (15.4)	
<i>Highest school degree Attained</i>				
None/Elementary school	655 (54.6)	326 (54.4)	329 (54.8)	0.622
Middle School	139 (11.6)	65 (10.8)	74 (12.4)	
High school/University	406 (33.8)	209 (34.8)	197 (32.8)	
<i>Occupation</i>				
Unemployed	220 (18.3)	105 (17.5)	115 (19.2)	0.097
Unskilled worker	290 (24.2)	132 (22.0)	158 (26.3)	
Skilled worker	690 (57.5)	363 (60.5)	327 (54.5)	
<b>Respondent (N=1,200)</b>				
<i>Gender</i>				
Male	397 (33.1)	202 (33.7)	195 (32.5)	0.668
Female	803 (66.9)	398 (66.3)	405 (67.5)	
<i>Age</i>				
18-34	320 (26.7)	152 (25.3)	168 (28.0)	0.296
≥ 35	880 (73.3)	448 (74.7)	432 (72.0)	
<i>Religion</i>				
Buddhist	1,196 (99.7)	598 (99.7)	598 (99.7)	1.000
Muslim/Christian	4 (0.3)	2 (0.3)	2 (0.3)	
<i>Years of schooling</i>				
0-7	616 (51.3)	306 (51.0)	310 (51.7)	0.872
8-14	414 (34.5)	211 (35.2)	203 (33.8)	
15-21	170 (14.2)	83 (13.8)	87 (14.5)	

**Table 19 (Continued)** Background characteristics of heads of household and respondents living in high and low dengue transmission areas.

Characteristics	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission	Low dengue transmission	
		(N=600)	(N=600)	
<i>Highest school degree Attained</i>				
None/Elementary school	616 (51.3)	306 (51.0)	310 (51.7)	0.971
Middle School	180 (15.0)	91 (15.2)	89 (14.8)	
High school/University	404 (33.7)	203 (33.8)	201 (33.5)	
<i>Occupation</i>				
Unemployed	333 (27.8)	172 (28.7)	161 (26.8)	0.211
Unskilled worker	635 (52.9)	324 (54.0)	311 (51.8)	
Skill worker	232 (19.3)	104 (17.3)	128 (21.4)	

\*Significantly different (P<0.05) with  $\chi^2$  test for two-independent samples.

**Table 20** Background characteristics and history of dengue infection of permanent household members living in high and low dengue transmission areas.

Characteristics	Total N=4,166	Number of person (%)		P-value
		High dengue transmission	Low dengue transmission	
		(N=2,246)	(N=1,920)	
<i>Gender</i>				
Male	1,891 (45.4)	1,037 (46.2)	854 (44.5)	0.274
Female	2,275 (54.6)	1,209 (53.8)	1,066 (55.5)	
<i>Age</i>				
0-34	2,107 (50.6)	1,166 (51.9)	941 (49.0)	0.062
≥ 35	2,059 (49.4)	1,080 (48.1)	979 (51.0)	
<i>Previous history of dengue infection</i>				
Yes	210 (5.0)	143 (6.4)	67 (3.5)	< 0.001
No	3,956 (95.0)	2,103 (93.6)	1,853 (96.5)	
<i>Last time of dengue infection (N=210)</i>				
≤ 6 months	27 (12.9)	21 (14.7)	6 (9.0)	0.248
> 6 months	183 (87.1)	122 (85.3)	61 (91.0)	
<i>Hospital visit/In patient care (N=210)</i>				
Yes	206 (98.1)	140 (97.9)	66 (98.5)	0.765
No	4 (1.9)	3 (2.1)	1 (1.5)	

\*Significantly different (P<0.05) with  $\chi^2$  test for two-independent samples.

Housing and basic services also are important socio-demographic variables responsible for dengue transmission. Simultaneously, each of 1,200 premises was carefully inspected, while the 1,200 respondents from each household were interviewed. Dataset of these housing and basic services also were used to test for significant differences in terms of determinants favoring dengue emergence.

Table 21 shows respective housing construction and basic utilities in a comparison between a high and low degrees of dengue transmission areas; there were significant differences in: main purpose of building, floor number and floor where household members live, construction material of house, number of household windows, window screen or window glass, backyard/open space attached to household, main water supply, frequency of emptying containers (large, medium, and small sizes), and solid waste and rubbish disposal.

**Table 21** Housing and basic services of households in high and low dengue transmission areas.

Housing and basic services	Total N=1,200	Number of households (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Main purpose of building</i>				
Residential	886 (73.8)	467 (77.8)	419 (69.8)	0.006
Business	298 (24.8)	127 (21.2)	171 (28.5)	
Restaurant	16 (1.4)	6 (1.0)	10 (1.7)	
<i>Number of floors of building</i>				
1	502 (41.8)	214 (35.7)	288 (48.0)	< 0.001
2	545 (45.4)	301 (50.2)	244 (40.7)	
3	137 (11.4)	76 (12.6)	61 (10.2)	
4	16 (1.4)	9 (1.5)	7 (1.1)	
<i>Floor where household members live (N=698)</i>				
1 <sup>st</sup>	221 (31.7)	132 (34.2)	89 (28.5)	0.013
2 <sup>nd</sup>	435 (62.3)	224 (58.0)	211 (67.6)	
3 <sup>rd</sup> and 4 <sup>th</sup>	42 (6.0)	30 (7.8)	12 (3.9)	
<i>Construction material of house</i>				
Concrete/stone/bricks	1,035 (86.3)	550 (91.7)	485 (80.8)	< 0.001
Wood	165 (13.8)	50 (8.3)	115 (19.2)	

**Table 21 (Continued)** Housing and basic services of households in high and low dengue transmission areas.

Housing and basic services	Total N=1,200	Number of households (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Years of residency</i>				
Residence time in this neighborhood				
0-9	453 (37.8)	221 (36.8)	232 (38.6)	0.134
10-19	257 (21.4)	140 (23.4)	117 (19.5)	
20-29	168 (14.0)	89 (14.8)	79 (13.2)	
30-39	105 (8.8)	59 (9.8)	46 (7.6)	
40-49	85 (7.1)	36 (6.0)	49 (8.2)	
50-59	75 (6.2)	32 (5.4)	43 (7.2)	
60-69	34 (2.8)	12 (2.0)	22 (3.7)	
≥ 70	23 (1.9)	11 (1.8)	12 (2.0)	
Residence time in this house				
0-9	556 (46.3)	272 (45.3)	284 (47.3)	0.204
10-19	271 (22.6)	144 (24.0)	127 (21.2)	
20-29	154 (12.8)	83 (13.8)	71 (11.8)	
30-39	84 (7.0)	46 (7.7)	38 (6.3)	
40-49	61 (5.1)	25 (4.2)	36 (6.0)	
50-59	48 (4.0)	19 (3.2)	29 (4.8)	
60-69	16 (1.3)	5 (0.8)	11 (1.8)	
≥ 70	10 (0.9)	6 (1.0)	4 (0.7)	
<i>Household windows</i>				
None	86 (7.2)	20 (3.3)	66 (11.0)	< 0.001
1-9	634 (52.8)	301 (50.2)	333 (55.5)	
10-19	330 (27.5)	198 (33.0)	132 (22.0)	
20-29	90 (7.5)	49 (8.2)	41 (6.8)	
30-39	44 (3.7)	25 (4.2)	19 (3.2)	
≥ 40	16 (1.3)	7 (1.1)	9 (1.5)	
<i>Screen/window glass of household (N=1,114)</i>				
Yes	719 (64.5)	444 (71.4)	305 (57.1)	< 0.001
No	395 (35.5)	166 (28.6)	229 (42.9)	
<i>Back yard/open space attached to household</i>				
Yes	828 (69.0)	473 (78.8)	355 (59.2)	< 0.001
No	372 (31.0)	127 (21.2)	245 (40.8)	

**Table 21 (Continued)** Housing and basic services of households in high and low dengue transmission areas.

Housing and basic services	Total N=1,200	Number of households (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Trees/bushes in back yard/open space (N=828)</i>				
None	374 (45.2)	215 (45.5)	159 (44.8)	0.287
1-4	296 (35.8)	179 (37.8)	117 (32.9)	
5-9	78 (9.4)	38 (8.0)	40 (11.3)	
10-14	42 (5.1)	24 (5.1)	18 (5.1)	
15-19	12 (1.4)	6 (1.3)	6 (1.7)	
≥ 20	26 (3.1)	11 (2.3)	15 (4.2)	
<i>Main water supply</i>				
Piped supply	1,074 (89.5)	537 (89.5)	537 (89.5)	< 0.001
Well water	49 (4.1)	46 (7.7)	3 (0.5)	
River water	26 (2.2)	12 (2.0)	14 (2.3)	
Canal/Rainwater	51 (4.2)	5 (0.8)	46 (7.7)	
<i>Storing water</i>				
Yes	1,122 (93.5)	559 (93.2)	563 (93.8)	0.640
No	78 (6.5)	41 (6.8)	37 (6.2)	
<i>Purpose for storing water</i>				
For washing (N=1,122)				
Yes	838 (74.7)	427 (76.4)	411 (73.0)	0.192
No	284 (25.3)	132 (23.6)	152 (27.0)	
For cleaning (N=1,122)				
Yes	691 (61.6)	338 (60.5)	353 (62.7)	0.442
No	431 (38.4)	221 (39.5)	210 (37.3)	
For drinking (N=1,122)				
Yes	65 (5.8)	30 (5.4)	35 (6.2)	0.542
No	1,057 (94.2)	529 (94.6)	528 (93.8)	
For all of these (N=1,122)				
Yes	209 (18.6)	97 (17.4)	112 (19.9)	0.274
No	913 (81.4)	462 (82.6)	451 (80.1)	
For cooking and others (N=1,122)				
Yes	46 (4.1)	29 (5.2)	17 (3.0)	0.067
No	1,076 (95.9)	530 (94.8)	546 (97.0)	

**Table 21 (Continued)** Housing and basic services of households in high and low dengue transmission areas.

Housing and basic services	Total N=1,200	Number of households (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Frequency of emptying containers</i>				
Large size container (N=1,122)				
Every day	7 (0.6)	3 (0.5)	4 (0.7)	0.015
Alternate	17 (1.5)	10 (1.8)	7 (1.2)	
Every week	101 (9.0)	40 (7.2)	61 (10.8)	
Every month	106 (9.5)	55 (9.8)	51 (9.1)	
Not usually every month	370 (33.0)	209 (37.4)	161 (28.6)	
Do not have	521 (46.4)	242 (43.3)	279 (49.6)	
Medium size container (N=1,122)				
Every day	40 (3.6)	10 (1.8)	30 (5.3)	0.001
Alternate	48 (4.3)	19 (3.4)	29 (5.2)	
Every week	231 (20.6)	102 (18.2)	129 (22.9)	
Every month	198 (17.6)	116 (20.8)	82 (14.6)	
Not usually every month	325 (29)	169 (30.2)	156 (27.7)	
Do not have	280 (24.9)	143 (25.6)	137 (24.3)	
Small size container (N=1,122)				
Every day	49 (4.4)	16 (2.9)	33 (5.9)	< 0.001
Alternate	36 (3.2)	16 (2.9)	20 (3.6)	
Every week	150 (13.4)	80 (14.3)	70 (12.4)	
Every month	99 (8.8)	69 (12.3)	30 (5.3)	
Not usually every month	137 (12.2)	67 (12.0)	70 (12.4)	
Do not have	651 (58.0)	311 (55.6)	340 (60.4)	
<i>Toilet that household uses</i>				
Toilet/Latrine inside	1,109 (92.4)	563 (93.8)	546 (91.0)	0.239
Toilet/Latrine outside	71 (6.0)	30 (5.0)	41 (6.8)	
Latrine shared by others	16 (1.3)	5 (0.8)	11 (1.8)	
Open air/No latrine	4 (0.3)	2 (0.4)	2 (0.4)	
<i>Solid waste and rubbish disposal</i>				
Collected by a man/a truck	788 (65.7)	416 (69.3)	372 (62.0)	0.044
Emptied in a particular site	386 (32.2)	170 (28.3)	216 (36.0)	
No fixed pattern	2 (0.2)	1 (0.2)	1 (0.2)	
Burning	24 (2.0)	13 (2.2)	11 (1.8)	

\*Significantly different (P<0.05) with  $\chi^2$  test for two-independent samples.

Because knowledge is a pivotal element influencing either positive or negative behaviors of humans, this study evaluated the knowledge of dengue among 1,200 respondents, as shown in Table 22. Almost all respondents in the two groups had heard about dengue, with television and health worker being their major first sources. However, there was a significant association ( $P$ -value $<0.001$ ) between the two groups of respondents and their second source of information. Another significant difference was observed in their opinion regarding the seriousness of the disease, with  $P$ -value=0.032. As for their knowledge about the spread of dengue, 3 out of the 7 ways of dengue transmission demonstrated significant associations between the two groups, including by mosquito, by direct contact, and by water, with  $P=0.015$ ,  $P=0.002$ , and  $P=0.006$ , respectively. More detailed answers were given about dengue's spread via mosquito by a significant higher number of respondents who lived in low dengue transmission areas than those living in high transmission areas.

**Table 22** Knowledge about dengue among respondents in high and low dengue transmission areas

Knowledge features	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Having heard about dengue</i>				
Yes	1,194 (99.5)	595 (99.2)	599 (99.8)	0.220
No	6 (0.5)	5 (0.8)	1 (0.2)	
<i>Two most important sources of dengue information (N=1,194)</i>				
First source				
Television	929 (77.8)	467 (78.5)	462 (77.1)	0.961
Radio	18 (1.5)	10 (1.7)	8 (1.3)	
Newspaper	12 (1.0)	6 (1.0)	6 (1.0)	
Health worker	164 (13.7)	80 (13.4)	84 (14.0)	
Friend/Peer	24 (2.0)	9 (1.5)	15 (2.5)	
Neighborhood	32 (2.7)	16 (2.7)	16 (2.7)	
Poster	8 (0.7)	4 (0.7)	4 (0.7)	
Hospital	7 (0.6)	3 (0.5)	4 (0.7)	

**Table 22 (Continued)** Knowledge about dengue among respondents in high and low dengue transmission areas.

Knowledge features	Total N=1,200	Number of person (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
<i>Second source</i>				
Radio	166 (13.9)	90 (15.1)	76 (12.7)	< 0.001
Newspaper	115 (9.6)	53 (8.9)	62 (10.4)	
Health worker	215 (18.0)	116 (19.5)	99 (16.5)	
Friend/Peer	102 (8.5)	62 (10.4)	40 (6.7)	
Neighborhood	151 (12.7)	93 (15.6)	58 (9.7)	
Poster	46 (3.9)	14 (2.4)	32 (5.3)	
Hospital	271 (22.7)	105 (17.7)	166 (27.7)	
Others	128 (10.7)	62 (10.4)	66 (11.0)	
<i>Suggestion received/Participated in training</i>				
Yes	665 (55.4)	323 (53.8)	342 (57.0)	0.270
No	535 (44.6)	277 (46.2)	258 (43.0)	
<i>Opinion about seriousness of the disease</i>				
Serious	1,130	553 (92.2)	557 (96.2)	0.032
Mild	29 (2.4)	20 (3.3)	9 (1.5)	
Maybe both	24 (2.0)	16 (2.7)	8 (1.3)	
Don't know	17 (1.4)	11 (1.8)	6 (1.0)	
<i>Spread of dengue</i>				
<i>Mosquito</i>				
Mentioned	1,135 (94.6)	558 (93.0)	577 (96.2)	0.015
Not mentioned	65 (5.4)	42 (7.0)	23 (3.8)	
<i>Direct contact</i>				
Mentioned	31 (2.6)	24 (4.0)	7 (1.2)	0.002
Not mentioned	1,169(97.4)	576 (96.0)	593 (98.8)	
<i>Water</i>				
Mentioned	31 (2.6)	23 (3.8)	8 (1.3)	0.006
Not mentioned	1,169 (97.4)	577 (96.2)	592 (98.7)	
<i>Flies</i>				
Mentioned	6 (0.5)	3 (0.5)	3 (0.5)	1.000
Not mentioned	1,194 (99.5)	597 (99.5)	597 (99.5)	

**Table 22 (Continued)** Knowledge about dengue among respondents in high and low dengue transmission areas.

Knowledge features	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N=600	Low dengue transmission N=600	
Dirty Environment				
Mentioned	31 (2.6)	18 (3.0)	13 (2.2)	0.363
Not mentioned	1,169 (97.4)	582 (97.0)	587 (97.8)	
Rats, birds, pigs, other animals				
Mentioned	4 (0.3)	1 (0.2)	3 (0.5)	0.616
Not mentioned	1,196 (99.7)	599 (99.8)	597 (99.5)	
Others				
Mentioned	18 (1.5)	12 (2.0)	6 (1.0)	0.154
Not mentioned	1,182 (98.5)	588 (98.0)	594 (99.0)	
<i>Perceived dengue could be prevented</i>				
Yes	1,146 (95.5)	571 (95.2)	575 (95.8)	0.785
No	20 (1.7)	10 (1.6)	10 (1.7)	
Uncertain/Don't know	34 (2.8)	19 (3.2)	15 (2.5)	

\*Significantly different ( $P < 0.05$ ) with  $\chi^2$  test for two-independent samples.

Not only is it important to know how much people understand about dengue transmission, it is also valuable to know to what extent people perceive vector-related knowledge and practices as the prerequisite for designing sustainable and effective vector control measures. Findings regarding vector-related knowledge and practices of respondents in high and low dengue transmission areas are presented in Table 23. Most of the respondents in the two groups knew how dengue mosquitoes develop, but the percentage of respondents who knew about this in high transmission areas was significantly lower than that observed in low transmission areas ( $P=0.001$ ). In addition, the percentage of respondents who knew correctly where dengue mosquitoes generally lay eggs was found to be higher in low dengue transmission areas than that of those in high dengue transmission areas. The other two parameters found to have a significant difference between the two groups of respondents were: having seen larvae in water and concern for having larvae in the household water

container, both of which demonstrated a higher percentage in the low dengue transmission areas than that in the other locality ( $P=0.028$  and  $P=0.030$ ).

Regarding anthropogenic activities against mosquitoes at the household level, research results revealed that the two mosquito-control practices of indoor spraying and putting larva-eating fish in water were observed to have significantly higher percentages of practice in high dengue transmission areas than those in low dengue transmission neighborhoods ( $P=0.039$  and  $P=0.033$ ). Regarding various activities conducted by the local government against mosquitoes, only the two activities of checking water containers and adding drugs or chemicals in water showed a higher percentage of use in high dengue transmission areas than that in low transmission areas ( $P=0.002$  and  $P=0.007$ ). In contrast, the percentages of spraying inside houses and fogging were much higher in high-endemic areas than in low-endemic ones ( $P=0.033$  and  $P=0.001$ ). Besides these meaningful government activities, the other two vector control activities that were observed to have significant differences in the percentage of practice between high and low dengue transmission areas (both  $P<0.001$ ) were: last time visit by health inspector and community efforts to clean the environment.

All respondents also were asked about vector control activities that household members and government staff should do. Only two household “should-do” activities (changing water once a week and eliminating stagnant water) demonstrated significantly higher percentages of practice in low dengue transmission areas than those in the high transmission locality ( $P=0.004$  and  $P=0.036$ ). Similarly, the two government should-do activities of checking the water containers and adding drugs or chemicals in water were found to have significantly higher percentages in low dengue transmission areas than those in high transmission areas, with  $P=0.049$  and  $P=0.027$ , respectively. There was however one government should-do activity (cutting plants) which demonstrated a significantly higher percentage in high dengue transmission areas than that in the low dengue transmission setting ( $P=0.039$ ).

**Table 23** Vector-related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
<i>From which mosquitoes develop</i>				
Eggs	46 (3.8)	19 (3.2)	27 (4.5)	0.011
Larvae	907 (75.6)	458 (76.3)	449 (74.8)	
Both eggs and larvae	198 (16.5)	88 (14.7)	110 (18.3)	
Others	12 (1.0)	8 (1.3)	4 (0.7)	
Don't know	37 (3.1)	27 (4.5)	10 (1.7)	
<i>Places where dengue mosquitoes generally lay their eggs &amp; breed</i>				
Clean water				
Mentioned	504 (42.0)	218 (36.3)	286 (47.7)	< 0.001
Not mentioned	696 (58.0)	382 (63.7)	314 (52.3)	
Dirty water				
Mentioned	408 (34.0)	202 (33.7)	206 (34.3)	0.807
Not mentioned	792 (66.0)	398 (66.3)	394 (65.7)	
Any water				
Mentioned	334 (27.8)	181 (30.2)	153 (25.5)	0.071
Not mentioned	866 (72.2)	419 (69.8)	447 (74.5)	
Wet clothes				
Mentioned	23 (1.9)	13 (2.2)	10 (1.7)	0.528
Not mentioned	1,177 (98.1)	587 (97.8)	590 (98.3)	
Plants				
Mentioned	43 (3.6)	23 (3.8)	20 (3.3)	0.641
Not mentioned	1,157 (96.4)	577 (96.2)	580 (96.7)	
Others				
Mentioned	716 (59.7)	377 (62.8)	339 (56.5)	0.025
Not mentioned	484 (40.3)	223 (37.2)	261 (43.5)	
Don't know				
Mentioned	13 (1.1)	11 (1.8)	2 (0.3)	0.012
Not mentioned	1,187 (98.9)	589 (98.2)	598 (99.7)	
<i>Having seen the larvae in water</i>				
Yes	1,159 (96.6)	572 (95.3)	587 (97.8)	0.028
No	34 (2.8)	21 (3.5)	13 (2.2)	
Uncertain	6 (0.5)	6 (1.0)	0 (0)	
Don't know	1 (0.1)	1 (0.2)	0 (0)	

**Table 23 (Continued)** Vector-related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
<i>Concern for having larvae in the household water container</i>				
Yes	1,138 (94.8)	561 (93.5)	577 (96.2)	0.030
No	24 (2.0)	11 (1.9)	13 (2.2)	
Uncertain	19 (1.6)	14 (2.3)	5 (0.8)	
Don't know	19 (1.6)	14 (2.3)	5 (0.8)	
<i>Activities at home to reduce the nuisance of mosquitoes</i>				
Nothing				
Yes	51 (4.2)	22 (3.7)	29 (4.8)	0.316
No	1,149 (95.8)	578 (96.3)	571 (95.2)	
Indoor spraying				
Yes	784 (65.3)	409 (68.2)	375 (62.5)	0.039
No	416 (34.7)	191 (31.8)	225 (37.5)	
Cleaning rubbish				
Yes	920 (76.7)	450 (75.0)	470 (78.3)	0.172
No	280 (23.3)	150 (25.0)	130 (21.7)	
Covering the water containers				
Yes	638 (53.2)	323 (53.8)	315 (52.5)	0.644
No	562 (46.8)	277 (46.2)	285 (47.5)	
Putting medicines in water				
Yes	654 (54.5)	326 (54.3)	328 (54.7)	0.908
No	546 (45.5)	274 (45.7)	272 (45.3)	
Putting fish in water				
Yes	273 (22.8)	152 (25.3)	121 (20.2)	0.033
No	927 (77.2)	448 (74.7)	479 (79.8)	
Killing mosquitoes				
Yes	634 (52.8)	317 (52.8)	317 (52.8)	1.000
No	566 (47.2)	283 (47.2)	283 (47.2)	
Personal protection with repellents				
Yes	173 (14.4)	91 (15.2)	82 (13.7)	0.460
No	1,027 (85.6)	509 (84.8)	518 (86.3)	

**Table 23 (Continued)** Vector-related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
<i>Activities of the government against mosquitoes</i>				
Nothing				
Mentioned	76 (6.3)	32 (5.3)	44 ((7.3)	0.155
Not mentioned	1,124 (93.7)	568 (94.7)	556 (92.7)	
Checking water containers				
Mentioned	212 (17.7)	86 (14.3)	126 (21.0)	0.002
Not mentioned	988 (82.3)	514 (85.7)	474 (79.0)	
Adding drugs/chemicals in water				
Mentioned	692 (57.7)	323 (53.8)	369 (61.5)	0.007
Not mentioned	508 (42.3)	277 (46.2)	231 (38.5)	
Spraying inside houses				
Mentioned	96 (8.0)	58 (9.7)	38 (6.3)	0.033
Not mentioned	1,104 (92.0)	542 (90.3)	562 (93.7)	
Educating people				
Mentioned	307 (25.6)	142 (23.7)	165 (27.5)	0.128
Not mentioned	893 (74.4)	458 (76.3)	435 (72.5)	
Spraying inside houses				
Mentioned	22 (1.8)	13 (2.2)	9 (1.5)	0.389
Not mentioned	1,178 (98.2)	587 (97.8)	591 (98.5)	
Giving fish to put in water				
Mentioned	10 (0.8)	4 (0.7)	6 (1.0)	0.525
Not mentioned	1,190 (99.2)	596 (99.3)	594 (99.0)	
Fogging				
Mentioned	938 (78.2)	492 (82.0)	446 (74.3)	0.001
Not mentioned	262 (21.8)	108 (18.0)	154 (25.7)	
Cutting plants				
Mentioned	18 (1.5)	13 (2.2)	5 (0.8)	0.057
Not mentioned	1,182 (98.5)	587 (97.8)	595 (99.2)	
Maintaining clean environment				
Mentioned	3 (0.2)	1 (0.2)	2 (0.3)	1.000
Not mentioned	1,197 (99.8)	599 (99.8)	598 (99.7)	

**Table 23 (Continued)** Vector-related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of person (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
<i>Last visit by health inspector</i>				
During last month	374 (31.2)	223 (37.2)	151 (25.2)	< 0.001
2-6 months ago	370 (30.8)	150 (25)	220 (36.7)	
7-12 months ago	65 (5.4)	33 (5.5)	32 (5.3)	
≥ 1 year	47 (3.9)	24 (4.0)	23 (3.8)	
Do not remember/Never	344 (28.7)	170 (28.3)	174 (29.0)	
<i>Having received support/materials to prevent dengue</i>				
Yes	932 (77.7)	466 (77.7)	466 (77.7)	1.000
No	268 (22.3)	134 (22.3)	134 (22.3)	
<i>Community effort to clean environment</i>				
Yes	513 (42.8)	284 (47.4)	229 (38.2)	< 0.001
No	527 (43.9)	227 (37.8)	300 (50.0)	
Don't know	160 (13.3)	89 (14.8)	71 (11.8)	
<i>Activities to get rid of dengue mosquitoes</i>				
Eliminate breeding places	397 (77.4)	215 (75.7)	182 (79.5)	0.228
Visit households	22 (4.3)	16 (5.6)	6 (2.6)	
Others	94 (18.3)	53 (18.7)	41 (17.9)	
<i>Household activities to prevent breeding places</i>				
Covering all water containers				
Yes	1,078 (89.8)	531 (88.5)	547 (91.2)	0.126
No	122 (10.2)	69 (11.5)	53 (8.8)	
Changing water once a week				
Yes	997 (83.1)	480 (80.0)	517 (86.2)	0.004
No	203 (16.9)	120 (20.0)	83 (13.8)	
Eliminating stagnant water				
Yes	1,018 (84.8)	496 (82.7)	522 (87.0)	0.036
No	182 (15.2)	104 (17.3)	78 (13.0)	
Brushing/Cleaning inner surface of water containers				
Yes	917 (76.4)	445 (74.2)	472 (78.7)	0.066
No	283 (23.6)	155 (25.8)	128 (21.3)	

**Table 23 (Continued)** Vector-related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of person (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
Using larva-eating fish				
Yes	676 (56.3)	335 (55.8)	341 (56.8)	0.727
No	524 (43.7)	265 (44.2)	259 (43.2)	
Putting medicines into containers				
Yes	993 (82.8)	485 (80.8)	508 (84.7)	0.079
No	207 (17.2)	115 (19.2)	92 (15.3)	
Removing larvae				
Yes	633 (52.8)	319 (53.2)	314 (52.3)	0.772
No	567 (47.2)	281 (46.8)	286 (47.7)	
Removing rubbish				
Yes	908 (75.7)	461 (76.8)	447 (74.5)	0.346
No	292 (24.3)	139 (23.2)	153 (25.5)	
Others				
Yes	0 (0)	0 (0)	0 (0)	NA
No	1,200 (100)	600 (100)	600 (100)	
<i>Dengue control activities that government should do</i>				
Checking water containers				
Mentioned	258 (21.5)	115 (19.2)	143 (23.8)	0.049
Not mentioned	942 (78.5)	485 (80.8)	457 (76.2)	
Adding drugs/chemicals to water				
Mentioned	678 (56.5)	320 (53.3)	358 (59.7)	0.027
Not mentioned	522 (43.5)	280 (46.7)	242 (40.3)	
Adding drugs/chemicals to water				
Mentioned	109 (9.1)	60 (10.0)	49 (8.2)	0.269
Not mentioned	1,091 (90.9)	540 (90.0)	551 (91.8)	
Educating people				
Mentioned	466 (38.8)	220 (36.7)	246 (41.0)	0.124
Not mentioned	734 (61.2)	380 (63.3)	354 (59.0)	
Supplying lid covers				
Mentioned	47 (3.9)	20 (3.3)	27 (4.5)	0.298
Not mentioned	1,153 (96.1)	580 (96.7)	573 (95.5)	

**Table 23 (Continued)** Vector related knowledge and practices of respondents in high and low dengue transmission areas.

Knowledge features and practices	Total N=1,200	Number of persons (%)		P-value
		High dengue transmission N = 600	Low dengue transmission N = 600	
Giving fish to put in water				
Mentioned	44 (3.7)	22 (3.7)	22 (3.7)	1.000
Not mentioned	1,156 (96.3)	578 (96.3)	578 (96.3)	
Fogging				
Mentioned	964 (80.3)	480 (80.0)	484 (80.7)	0.771
Not mentioned	236 (19.7)	120 (20.0)	116 (19.3)	
Cutting plants				
Mentioned	48 (4.0)	31 (5.2)	17 (2.8)	0.039
Not mentioned	1,152 (96.0)	569 (94.8)	583 (97.2)	
Others				
Mentioned	73 (6.1)	30 (5.0)	43 (7.2)	0.116
Not mentioned	1,127 (93.9)	570 (95.0)	557 (92.8)	
Nothing				
Mentioned	50 (4.2)	26 (4.3)	24 (4.0)	0.773
Not mentioned	1,150 (95.8)	574 (95.7)	576 (96.0)	

\*Significantly different (P<0.05) with  $\chi^2$  test for two-independent samples.

#### **4.4.2 Stakeholder analysis**

By applying a stakeholder analysis matrix form, a total of 32 various stakeholders associated with the dengue problem and vector control activities in the study area were recruited and individually interviewed with regards to functions and interests. Soon after, the stakeholder dataset (mainly obtained from interviewing and a set of published and unpublished sources) was transformed into stakeholder information; stakeholder identification was in turn performed using the checklist method. Three criteria consisting of type, organization, and mission of stakeholder were utilized for the identification, and the results are shown in Table 24. The types of stakeholders included 21 agencies, 8 groups, and 3 individuals. Organization stakeholders were categorized as 17 government organizations, 12 non-government structures, and 3 state enterprises. When the mission or main function was applied, these organizational stakeholders then were classified more specifically as 15 responsible directly for public health, 8 for community service, 1 for public service, 2 for garbage disposal, 5 for water supply, and 1 for urban planning.

**Table 24** Identification and description of 32 participating stakeholders involved in the problem of dengue and vector control in the study area.

ID	Stakeholder	Type	Organization	Mission
1	Provincial Public Health Office	Agency	Government	Public Health
2	District Public Health Office-1	Agency	Government	Public Health
3	District Public Health Office-2	Agency	Government	Public Health
4	General Hospital	Agency	Government	Public Health
5	Community Hospital-1	Agency	Government	Public Health
6	Community Hospital-2	Agency	Government	Public Health
7	Health Center	Agency	Government	Public Health
8	Private Hospital	Agency	Non-Government	Public Health
9	Community Leaders-1	Group	Non-Government	Community Service
10	Community Leaders-2	Group	Non-Government	Community Service
11	Community Leaders-3	Group	Non-Government	Community Service
12	Community Leaders-4	Group	Non-Government	Community Service
13	Community Leaders-5	Group	Non-Government	Community Service
14	Community Leaders-6	Group	Non-Government	Community Service
15	Community Leaders-7	Group	Non-Government	Community Service
16	Community Leaders-8	Group	Non-Government	Community Service
17	Provincial Administrative Organization	Agency	Government	Public Service
18	Division of PH and Environment-1	Agency	Government	Public Health
19	Division of PH and Environment-2	Agency	Government	Public Health
20	Division of PH and Environment-3	Agency	Government	Public Health
21	Division of PH and Environment-4	Agency	Government	Public Health
22	Division of PH and Environment-5	Agency	Government	Public Health
23	Division of PH and Environment-6	Agency	Government	Public Health
24	Division of PH and Environment-7	Agency	Government	Public Health
25	Sanitary Works Division	Agency	Government	Garbage Disposal
26	Garbage Management Company	Individual	Non-Government	Garbage Disposal
27	Waterworks Division	Agency	Government	Water Supply
28	Waterworks Authority-1	Agency	State Enterprise	Water Supply
29	Waterworks Authority-2	Agency	State Enterprise	Water Supply
30	Water Supply Company-1	Individual	Non-Government	Water Supply
31	Water Supply Company-2	Individual	Non-Government	Water Supply
32	Public Works and Urban Planning	Agency	Government	Urban Planning

Beyond the identification process, the stakeholder analysis included profiling and positioning all of respective stakeholders, with the Social Analysis CLIP method being applied to create profiles of all relevant parties involved in the dengue problem. Four main components constituting this method include power, interests, legitimacy, and existing relations of collaboration and conflict.

The first component, power, is the ability to influence others and achieve a goal using the resources that one stakeholder controls. The 4 major sources of power are 1) economic wealth, 2) political authority, 3) the ability to use force or threats of force, 4) access to information (knowledge and skill) and the means to communicate its message. The rating scale of individual sources of power was separately determined based on how important the stakeholders are with respect to the dengue problem and related activities, the maximum scores being 9 for economic wealth, 5 for political authority, 3 for the ability to use force or threats of force, and 7 for access to information along with ways to communicate. The determination of the power level of each stakeholder was performed in accordance with their total scores in comparison with the maximum total, which was 24. As a result, three levels of power were rated as follows: 1) low level, with represented scores 0-6 (0-29%), 2) middle level, with represented scores 7-17 (29-73%), and 3) high level, with represented scores 18-24 (73-100%).

The net interests of each stakeholder is the second component and are the gains that one makes from the situation or proposed action minus the estimated losses. The 4 principal categories of gains and losses resulting from the dengue problem and vector control action consist of 1) economic wealth, 2) political authority, 3) time, and 4) social ties. To obtain the overall value of gains and losses for respective stakeholders, each of the 4 categories of gains and losses was individually evaluated based upon the maximum range of values (+10 to -10) and the probability (0-100%) and then all values of each of 4 categories were totaled accordingly. The net results were classified into 5 levels, namely: 1) high gains, which stood for values 20-40, 2) middle gains, which stood for values 0.1-19, 3) low/no interests, represented by a zero score, 4) middle losses, which stood for values (-0.1) to (-19), and 5) high net losses, which stood for values (-20) to (-40).

The third component, legitimacy is the degree to which other parties recognize the stakeholders’ rights and responsibilities (exercised with resolve) and rank them high. It helps us gain an insight into stakeholder legitimacy, using the three “R” factors: rights, responsibilities, and resolve. Because the three “R” factors are not equally important, different ranges of values were set: 1-9 for right (R1), 1-6 for responsibilities (R2), and 1-4 for resolve (R3). In this case, the total legitimacy score was derived from the multiplication between value<sub>1</sub> (R1 x R3) and value<sub>2</sub> (R2 x R3), the maximum total attained being 864. The legitimacy score totals were then categorized into 3 levels as follows: 1) low level, signified by scores 1-23, 2) middle level, signified by scores 24-603, and 3) high level, signified by scores 604-864.

Finally, the individual levels of these 3 components were employed to categorize each stakeholder, and the stakeholder was given the letter P, the letter I, or the letter L only if the corresponding score was high or middle. The entire 7 stakeholder categories resulting from the process of stakeholder profiling and positing are summarized in Table 25.

**Table 25** Stakeholder categories vertically ranked in accordance with the 3 main factors of power (P), interests (I), and legitimacy (L).

Categories	High/Middle Ratings	Low/No Ratings
<b>UPPER</b>		
Dominant	PIL Power, Interest (+ or -), Legitimacy	
Forceful	PI Power, Interest (+ or -)	Legitimacy
<b>MIDDLE</b>		
Influential	PL Power, Legitimacy	Interest (+ or -)
Dormant	P Power	Legitimacy, Interest (+ or -)
Respected	L Legitimacy	Power, Interest (+ or -)
<b>LOWER</b>		
Vulnerable	IL Interest (+ or -), Legitimacy	Power
Marginalized	I Interest (+ or -)	Power, Legitimacy

The last component (existing relations of collaboration and conflict) indicates the particular ties that each stakeholder has with other stakeholders. Three aspects were taken into consideration, covering the strength and direction of the influence of one group on another, along with whether the type of influence is positive or negative.

Table 26 and Figure 51 summarize all 32 stakeholder profiles that were created based on the three factors of power, interests, and legitimacy. Table 2 shows how each stakeholder was classified into 7 categories, namely: dominant, forceful, influential, dormant, respected, vulnerable, and marginalized. Of all the categories, only 4 (dominant, influential, forceful, and marginalized) were observed among all the respective stakeholders. There were 20 stakeholders considered as dominant, including 1 provincial public health office, 2 district public health offices, 1 health center, 8 groups of community leaders, 1 provincial administration organization, and 7 divisions of public health and environment (belong to municipality offices). Marginalized was the second category of 6 stakeholders, which is comprised of 1 waterworks division, 2 waterworks authorities, 2 water supply companies, and 1 public works and urban planning office. Three stakeholders were categorized as influential, including 1 general hospital and 2 community hospitals. The last 3 stakeholders, classified as forceful, were 1 private hospital and 1 sanitary work division (belong to the municipality office), and 1 garbage management company.

**Table 26** Stakeholder profiling and positioning by using Social Analysis CLIP.

ID	Stakeholder	Group <sup>a</sup>	Power		Legitimacy		Interest		Stakeholder Category	
			Score	Level	Score	Level	Score	Net <sup>b</sup>		
1	Provincial Public Health Office	PPHO	21	H	864	H	2.5	+	PIL	Dominant
2	District Public Health Office-1	DPHO	20	H	864	H	2.5	+	PIL	Dominant
3	District Public Health Office-2	DPHO	20	H	864	H	2.5	+	PIL	Dominant
4	General Hospital	GH	17	M	252	M	0	0	PL	Influential
5	Community Hospital-1	CH	15	M	315	M	0	0	PL	Influential
6	Community Hospital-2	CH	15	M	315	M	0	0	PL	Influential
7	Health Center	HC	17	M	864	H	3.9	+	PIL	Dominant
8	Private Hospital	PH	11	M	21	L	-7.5	-	PI	Forceful
9	Community Leaders-1	CL	11	M	180	M	2.4	+	PIL	Dominant
10	Community Leaders-2	CL	10	M	180	M	2.4	+	PIL	Dominant
11	Community Leaders-3	CL	11	M	180	M	2.4	+	PIL	Dominant
12	Community Leaders-4	CL	10	M	180	M	2.4	+	PIL	Dominant
13	Community Leaders-5	CL	11	M	180	M	2.4	+	PIL	Dominant
14	Community Leaders-6	CL	11	M	180	M	2.4	+	PIL	Dominant
15	Community Leaders-7	CL	10	M	180	M	2.4	+	PIL	Dominant
16	Community Leaders-8	CL	11	M	180	M	2.4	+	PIL	Dominant

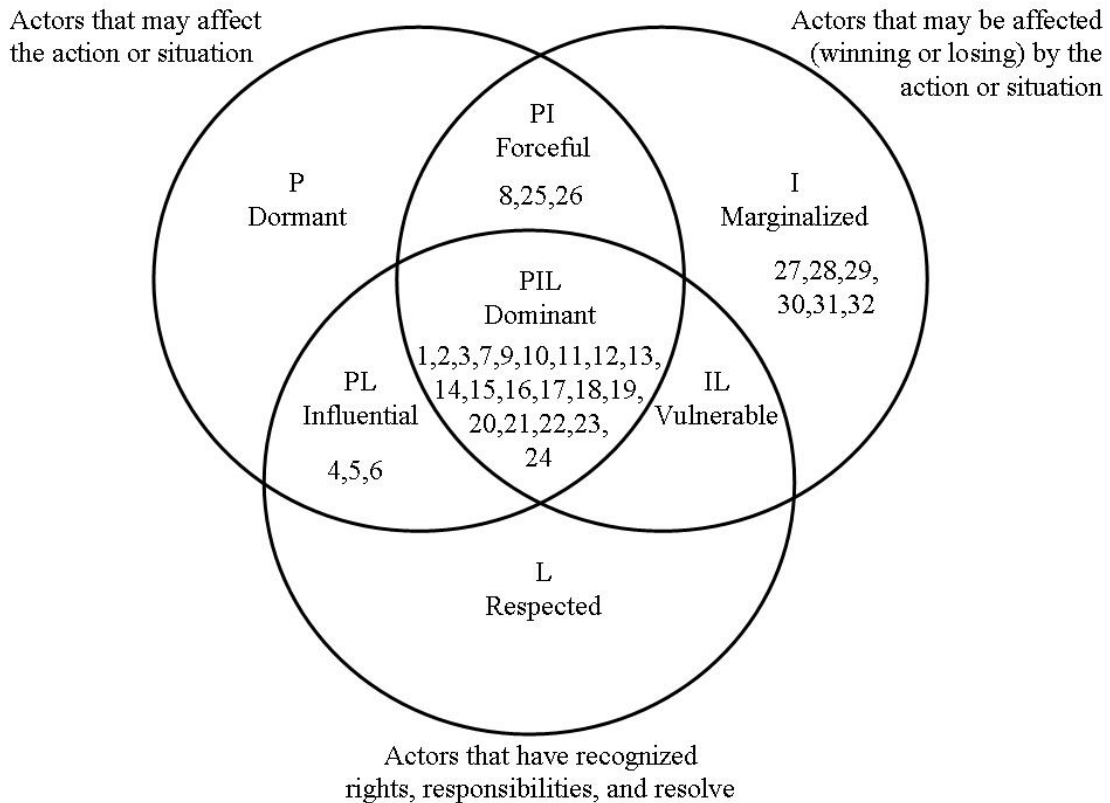
<sup>a</sup> Stakeholder Groups: PPHP=Provincial Public Health Office, DPHO= Provincial Public Health Office, G/C/PH=General/Community/Private Hospital, CL=Community Leader, PAO=Public Service, DPHE= Division of PH and Environment, GD=Garbage Disposal, WSG=Water Supply (Government)

**Table 26 (Continued)** Stakeholder profiling and positioning by using Social Analysis CLIP.

ID	Stakeholder	Group <sup>a</sup>	Power		Legitimacy		Interest		Stakeholder Category	
			Score	Level	Score	Level	Score	Net <sup>b</sup>		
17	Provincial Administrative Organization	PAO	20	H	640	H	8	+	PIL	Dominant
18	Division of PH and Environment-1	DPHE	18	H	512	M	10.5	+	PIL	Dominant
19	Division of PH and Environment-2	DPHE	16	M	512	M	10.5	+	PIL	Dominant
20	Division of PH and Environment-3	DPHE	16	M	512	M	10.5	+	PIL	Dominant
21	Division of PH and Environment-4	DPHE	15	M	512	M	10.5	+	PIL	Dominant
22	Division of PH and Environment-5	DPHE	15	M	512	M	10.5	+	PIL	Dominant
23	Division of PH and Environment-6	DPHE	15	M	512	M	10.5	+	PIL	Dominant
24	Division of PH and Environment-7	DPHE	15	M	512	M	10.5	+	PIL	Dominant
25	Sanitary Works Division	SWD	9	M	1	L	4	+	PI	Forceful
26	Garbage Management Company	GMC	6	L	1	L	2.6	+	PI	Forceful
27	Waterworks Division	WSG	4	L	1	L	2.6	+	I	Marginalized
28	Waterworks Authority-1	WSG	4	L	1	L	2.3	+	I	Marginalized
29	Waterworks Authority-2	WSG	4	L	1	L	2.3	+	I	Marginalized
30	Water Supply Company-1	WSC	4	L	1	L	-5.1	-	I	Marginalized
31	Water Supply Company-2	WSC	4	L	1	L	-5.1	-	I	Marginalized
32	Public Works and Urban Planning	PWUP	5	L	6	L	-0.2	-	I	Marginalized

<sup>a</sup> Stakeholder Groups: WSC=Water Supply (Company), PWUP=Public Works and Urban Planning

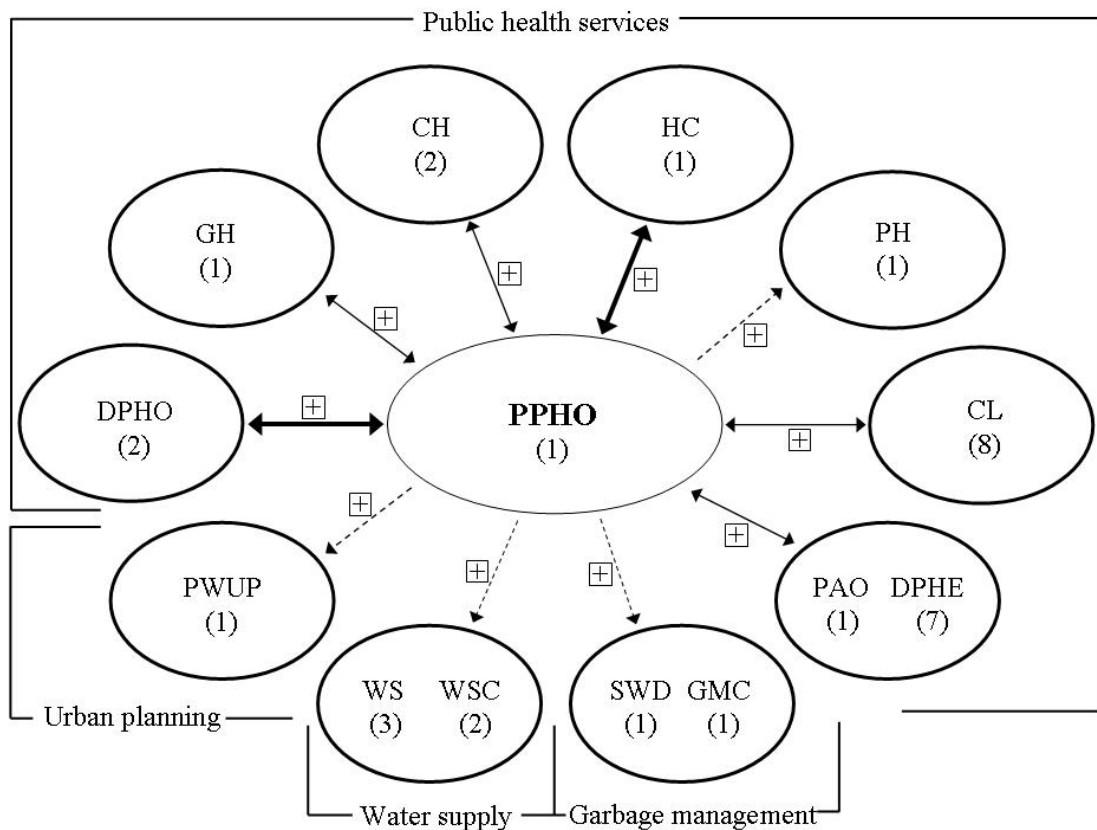
<sup>b</sup> Net Interests: (++) = High net gains, (+) = Middle net gains, (0) = Low/No interests, (-) = Middle net losses, (--) = High net losses



**Figure 51** A Venn diagram elucidating stakeholder profiles as a result of an analysis based on Power (P), Interests (I), and Legitimacy (L).

Apart from the earlier three main components used for creating the stakeholder profiles, existing relations of collaboration and conflict is an important component for obtaining insight into the types and levels of collaboration and potential conflicts between one stakeholder and another. In Figure 52, a Venn diagram is used to depict who the stakeholders were in association with the provincial public health office (PPHO). First, all stakeholders were regrouped by considering their mission basis, which resulted in 4 groups: public health services, garbage management, water supply, and urban planning. A majority of stakeholders were mainly responsible for public health services. Two stakeholders were responsible for garbage management. Five stakeholders had water supply as their main duty. Only one stakeholder was found to have a responsibility for urban planning.

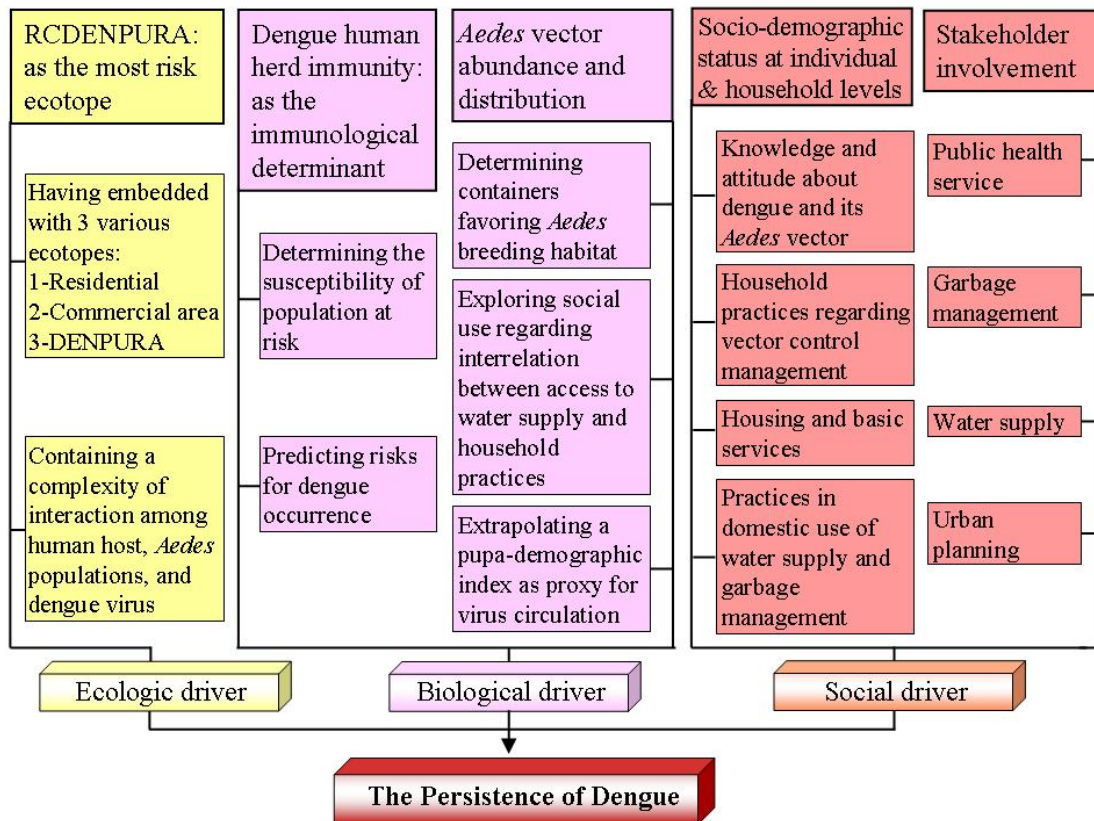
By holding the provincial public health office (PPHO) as the center, all other 31 stakeholders had some positive influence on it and vice versa. Except for PH, all of stakeholders responsible for public health services had a two-way relationship with PPHO. There was only a one-way relationship from PPHO to all 9 stakeholders, who were mainly responsible for garbage management, water supply, and urban planning. Only 2 groups of DPHO and HC contained a robust relationship with PPHO and exhibited it in both directions. The 4 groups of GH, CH, CL, and PAO/DPHE revealed a fair level of two-way ties with PPHO. The weakest degree of one-way relationship with PPHO was observed in the following 4 groups: PH, SWD/GMC, WS/WSC, and PWUP.



**Figure 52** A Venn diagram portraying who the stakeholders are according to the provincial public health office (PPHO). Each stakeholder group is represented by a circle, and arrows are used to indicate the relationship with the PPHO. The thickness and direction of the line stands for the strength and direction of the influence of one group on another. The plus and minus signs signify whether that influence is positive or negative.

Ultimately, a contextualized determination of the local eco-bio-social system, based on various lines of research evidence, was summarized in terms of cardinal drivers that caused the persistence of dengue in urban-and semi-urban study areas, and these empirically evident findings were evaluated with regards to causes and effects, as shown in Figure 53.

According to a series of research findings obtained, all of the significant determinants that greatly contributed to the dengue and vector control problem in the study area can be classified into three major groups: 1) Ecologic driver, 2) Biological driver, and 3) Social driver. For the ecological driver, research results clearly indicated that the ecotope of residential mixed with commercial area and densely populated urban residential area or RCDENPURA played a pivotal role in dengue transmission, while the other three ecotopes (commercial area, DENPURA, and RC) did not contribute to either the higher *Aedes* mosquito density or an increase in dengue incidence. The dengue human herd immunity and *Aedes* mosquito abundance that constituted the biological driver also affirmed their important roles in the emergence of dengue. Speaking specifically, at the local community level, a higher proportion rate of susceptible humans in the population than immune persons was considered a risk for disease outbreak, while a great number of *Aedes* mosquitoes and their geographical expansion were the consequential outcomes of more abundant artificial water-holding containers created mainly by indigenous household members. The socio-demographic determinants, as well as factors related to public health service, garbage management, water supply, and urban planning also contributed significantly to the persistence of dengue. A lack of knowledge and desirable attitude regarding dengue and its vector in conjunction with poor and/or improper water-supply practices and garbage management at either individual or household levels have obviously become part of the social driver; while on the other hand, all respective stakeholders in this local ecosystem were somehow involved in different degrees with the dengue and vector control problem.



**Figure 53** A Tributary Causal diagram elucidating various sets of empirical eco-bio-social drivers responsible for the persistence of dengue in urban-and semi-urban ecosystem settings.

#### **4.5 A Comprehensive Integrated Investigation of Ecological and Socio-demographic Determinants as Epidemiological Risk Factors for Dengue**

Beyond the previous individual-focused examinations of ecologic and socio-demographic parameters underlying dengue occurrence, all of these ecological and social variables obtained from the survey were investigated for determination of dengue transmission risk. A series of two association tests were applied: a univariate logistic regression and multivariate regression model. By the use of the first model, a set of socio-demographic variables (including knowledge, attitude, and practices of respondents, as well as household and government, and housing with basic services) were tested among each of the 4 ecotopes in order to determine which ones became underlying risk factors in a certain ecotope. For the latter model, only the residential mixed with commercial area and RCDENPURA (densely populated urban residential area) ecotopes—which previously were considered as significant risk—were further used to investigate the association between the significant socio-demographic variables for dengue transmission obtained from the preceding step.

In Table 27, results of the univariate logistic analyses of socio-demographic factors associated with the dengue emergence are shown per ecotope. These determinants for the occurrence of dengue differed substantially among all subjective ecotopes. For the ecotope of DENPURA, research findings revealed a number of determinants that significantly predicted risk for dengue transmission. Females were twice more likely to get dengue infection than males (OR=2.000, P=0.225). Persons who had a history of movement during the last 3 months had an increased risk of dengue infection than those who did not (OR=2.750, P=0.091). A significant increase in risk was also observed in two other determinants: families that had a higher average income (>4,499 Baht per month) and family members who stayed at their home more than 10 years (OR=3.656, P=0.071 and OR=3.366, P=0.094). With regards to the dengue knowledge, attitudes, and practices related to dengue vector control among the respondents, some predictors belonging to these three main determinants revealed an association with dengue emergence. People who had lower knowledge about *Aedes* mosquito breeding places were more likely to get

a dengue infection than those who had higher levels of knowledge (OR=1.696, P=0.323). Persons with lower concern about the seriousness of dengue had twice the risk as those with high concern (OR=2.750, P=0.091). Among all determinants of dengue vector control practices, only 3 variables (emptying medium-size water containers irregularly, fogging, and community effort to clean the environment) were observed to become a significant predictor for dengue transmission (OR=1.771, P=0.295; OR=1.946, P=0.390; OR=1.737, P=0.257). Since factors at the contextual and environmental level in which the individual is embedded can be significant determinants, some important predictors of housing and basic services were tested. Findings regarding these predictors demonstrated that there was a significant association between the house attachment and the occurrence of dengue (OR=1.519, P=0.587).

Some significant variables associated with dengue transmission were observed in the commercial area ecotope. Females dwelling in this kind of ecosystem were likely to have twice the risk of males (OR=2.225, P=0.121). With regards to occupation as risk, unemployed persons had a higher risk of dengue infection compared to other occupation groups (OR=8.000, P=0.03). Persons with a history of movement during the last 3 months, as well as families with a higher income, also posed an increased risk of dengue infection (OR=2.651, P=0.153 and OR=2.417, P=0.060). Concerning dengue knowledge, respondents who misconceived about the spread of dengue, as well as the group with a lower overall knowledge about dengue, were strongly associated with dengue infection (OR=3.997, P=0.232 and OR=7.242, P=0.002). Two aspects involving lower attitude demonstrated a significant predictor for dengue emergence: gender role and government role (both ORs=3.656 and both Ps=0.004). Regarding the respondents' vector control behaviors, dwellers who stored water had twice the risk than those who did not store water (OR=2.143, P=0.003), while irregularly emptying small-size water containers and community effort to clean the environment were also good predictors for the transmission of dengue (OR=7.614, P=0.002 and OR=1.843, P=0.196). The only significant socio-demographic predictor with regards to housing and basic services was the main purpose of housing; specifically, it was revealed that houses used mainly for residency posed a higher risk than those utilized for business or restaurants (OR=2.789, P=0.035).

In similar fashion, all the same socio-demographic predictors were determined for the RCDENPURA ecotope and results were explained as follows. Females had a higher risk of dengue infection compared to males (OR=2.716, P=0.022). People who had a history of movement during the last 3 months, as well as persons who had higher years of schooling ( $\geq 5$  years), were more likely to get dengue infection than those who did not have a history and persons who received fewer years of schooling (OR=2.005, P=0.216 and OR=1.604, P=0.214). Families earning 0-4,499 baht monthly were found to be 1.833 times more likely to be infected with dengue than those earning more than 4,500 baht per month (OR=1.833, P=0.103).

For variables concerning dengue knowledge, attitudes, and practices related to dengue and vector control activities, some of the variables were found to possess dengue infection risk. Dengue prevalence was greater amongst respondents who had insufficient knowledge about dengue spread compared to those who clearly understood the means of dengue transmission (OR=2.129, P=0.122). In addition, respondents who possessed a low level of overall dengue knowledge were found to have a risk higher than those who possessed a high level (OR=2.512, P=0.049).

With regards to attitude determinants, respondents with lower attitudes regarding family role and/or government role exhibited a greater risk for dengue infection than those with high attitudes (both ORs = 1.640 and both Ps = 0.167). Practices concerning the emptying of both large-size and medium-size containers irregularly were observed to become significant predictors for the occurrence of dengue (OR=1.920, P=0.231 and OR=1.650, P=0.226). As for practices related to the reduction of mosquito nuisance, two variables (applying repellent and fogging) were also found to be significant predictors for dengue emergence (OR=7.319, P=0.026 and OR=3.967, P=0.019). Among all the variables related to housing and basic services, two predictors (floor number and having a backyard) were observed to possess a risk for dengue transmission. Houses with a one floor had a greater probability of dengue emergence than those with  $\geq 2$  floors (OR=2.008, P=0.070). Houses having a backyard were also found to possess a greater risk for dengue transmission than those having no backyard (OR=1.958, P=0.185).

For the ecotope of RC, a few socio-demographic determinants were likely to become a good predictor. These significant predictors included years of schooling

of respondents, attitude about dengue seriousness, household practice in emptying large-size water containers, and houses with  $\geq 10$  windows. Respondents with  $\geq 5$  years of schooling had a greater probability to getting dengue infection than those with  $< 5$  years of schooling (OR=1.523, P=0.106). Persons who considered dengue not serious were likely to possess a greater risk for dengue infection than those who considered dengue serious (OR=1.699, P=0.259). In addition, the practice of irregularly emptying the large-size water containers was observed to be a good predictor for dengue transmission (OR=1.782, P=0.296). The significant predictor regarding housing and basic services was that houses with  $\geq 10$  windows posed a risk higher than those with  $< 10$  windows (OR=2.252, P=0.001).

**Table 27** Univariate logistic analyses of socio-demographic determinants for dengue transmission among four different ecotopes.

Socio-demographic predictors	DENPURA			Commercial area			RCDENPURA			RC		
	N	OR	P-value	N	OR	P-value	N	OR	P-value	N	OR	P-value
<b>Respondent background</b>												
Sex												
Male	64	1		70	1		67	1		196	1	
Female	136	2.000	0.225	130	2.225	0.121	133	2.716	0.022*	404	0.926	0.758
Age (years)												
≥ 35	162			136	1		149	1		433	1	
18-34	38	1.074	0.094	64	1.152	0.761	51	1.188	0.666	167	1.085	0.755
Occupation												
Skilled workers	55	1		13	1		43	1		121	1	
Unskilled workers	64	0.692	0.651	177	1.443	0.730	92	1.139	0.782	302	1.031	0.923
Unemployed	81	1.021	0.971	10	8.000	0.063	65	0.991	0.085	177	1.079	0.824
Movement during last 3 months												
No	181	1		188	1		184	1		568	1	
Yes	19	2.750	0.091	12	2.651	0.153	16	2.005	0.216*	32	1.181	0.740
Years of schooling												
0-4	94	1		35	1		79	1		216	1	
≥ 5	106	0.729	0.509	165	1.009	0.988	121	1.604	0.214*	384	1.523	0.106
Highest school degree												
Elementary and lower	55	1		145	1		120	1		296		
Middle and higher	145	1.417	0.511	55	0.867	0.792	80	1.054	0.884	304	1.086	0.730
Average family income (baht)												
≥ 4500	54	1		160	1		90	1		342	1	
0-4499	146	3.656	0.071	40	2.417	0.060	110	1.833	0.103*	258	0.877	0.587
Years of stay in house												
< 10	51	1		118	1		109	1		278	1	
≥ 10	149	3.366	0.094	82	0.742	0.519	91	0.798	0.532	322	1.187	0.476

**Table 27 (Continued)** Univariate logistic analyses of socio-demographic determinants for dengue transmission among four different ecotopes.

Socio-demographic predictors	DENPURA			Commercial area			RCDENPURA			RC		
	N	OR	P-value	N	OR	P-value	N	OR	P-value	N	OR	P-value
<b>Dengue knowledge</b>												
Dengue spread												
Conceive	173	1		197	1		178	1		517	1	
Misconceive	27	0.312	0.241	3	3.977	0.232	22	2.129	0.122	83	1.079	0.821
Breeding place												
High	70	1		71	1		75	1		159	1	
Low	130	1.696	0.323	129	1.036	0.939	125	1.089	0.818	441	0.697	0.156
Overall knowledge												
High	167	1		191	1		177	1		518	1	
Low	33	1.302	0.657	9	7.242	0.002	23	2.512	0.049*	82	0.752	0.445
<b>Attitude about vector control</b>												
Gender role												
High	91	1		56	1		81	1		284	1	
Low/Fair	105	1.625	0.320	144	0.698	0.441	119	0.343	0.003	316	1.047	0.846
Family role												
Fair	96	1		125	1		107	1		366	1	
Low	104	0.915	0.850	75	3.656	0.004	93	1.640	0.167	234	1.126	0.623
Government role												
High	96	1		125	1		107	1		366	1	
Low/Fair	104	0.915	0.850	75	3.656	0.004	93	1.640	0.167	234	1.126	0.623
Seriousness of dengue												
High	181	1		194	1		184	1		571	1	
Not serious/Low	19	2.750	0.091	6	1.135	0.370	16	0.949	0.937	29	1.699	0.259

**Table 27 (Continued)** Univariate logistic analyses of socio-demographic determinants for dengue transmission among four different ecotopes.

	DENPURA			Commercial area			RCDENPURA			RC		
	N	OR	P-value	N	OR	P-value	N	OR	P-value	N	OR	P-value
<b>Socio-demographic predictors</b>												
<b>Household practices in vector control</b>												
Main water supply												
Well/river/others	58	1		0			3	1		65	1	
Piped supply	142	0.577	0.253	200	N/A	N/A	197	0.802	0.390	535	1.351	0.471
Storing water												
No	1	1		32	1		5	1		40	1	
Yes	199	0.095	0.003	168	2.143	0.310	195	0.968	0.977	560	0.516	0.092
Emptying the water container												
Large size container												
Regularly	118			69	1		78	1		211	1	
Irregularly	20	N/A	0.214	21	0.806	0.738	37	1.920	0.231	47	1.782	0.296
Medium size container												
Regularly	104	1		24	1		85	1		310	1	
Irregularly	67	1.771	0.295	41	1.029	0.971	70	1.650	0.226	141	1.292	0.409
Small size container												
Regularly	28	1		26	1		53	1		129	1	
Irregularly	34	0.593	0.673	65	7.614	0.002	44	0.614	0.331	92	1.161	0.727
Reducing mosquito nuisance												
Indoor spraying												
No	90	1		79	1		80	1		167	1	
Yes	110	0.629	0.343	121	0.638	0.344	120	0.923	0.827	433	1.325	0.268
Killed by chemical												
No	65	1		154	1		73	1		274	1	
Yes	135	0.336	0.078	46	0.513	0.153	127	0.968	0.931	326	0.869	0.559

**Table 27 (Continued)** Univariate logistic analyses of socio-demographic determinants for dengue transmission among four different ecotopes.

Socio-demographic predictors	DENPURA			Commercial area			RCDENPURA			RC	
	N	OR	P-value	N	OR	P-value	N	OR	P-value	N	P-value
Applying repellent											
No	170	1		191	1		173	1		493	
Yes	30	1.000	1.000	9	0.234	0.036	27	7.319	0.026*	107	0.965
Temephos (Abate) Sand											
No	43	1		137	1		85	1		243	
Yes	157	0.377	0.187	63	0.845	0.719	115	0.618	0.197	357	0.826
Indoor spraying											
No	196	1		199			193			516	
Yes	4	N/A	1.000	1	N/A	0.115	7	N/A	0.349	84	0.942
Educating people											
No	149	1		191	1		162	1		391	
Yes	51	1.414	0.552	9	0.432	0.302	138	1.090	0.852	209	1.103
Fogging											
Yes	166	1		156	1		157	1		459	
No	34	1.946	0.380	44	0.604	0.299	43	3.967	0.019	141	1.203
Government support											
No	15			72	1		46	1		133	
Yes	185	N/A	0.371	128	0.456	0.130	154	0.684	0.403	467	1.248
<b>Community effort by cleaning environment</b>											
No	106	1		114	1		131	1		336	
Yes	94	1.737	0.257	86	1.843	0.196	69	0.927	0.838	264	1.267
<b>Housing and basic services</b>											
House attachment											
Separated	28	1		3	1		33	1		159	

Attached	172	1.519	0.587	197	0.883	0.529	167	0.712	0.452	441	0.795	0.379		
<b>Table 27 (Continued)</b> Univariate logistic analyses of socio-demographic determinants for dengue transmission among four different ecotopes.														
Socio-demographic predictors	DENPURA						RCDENPURA						RC	
	N	OR	P-value	N	OR	P-value	N	OR	P-value	N	OR	P-value		
<b>Main purpose of housing</b>														
Business/Restaurant	2	1	169	1	1	4	1	138	1					
Residential	198	0.899	0.636	31	2.789	0.035	196	0.722	0.779	462	0.848	0.546		
<b>Floor number</b>														
≥ 2	93	1	166	1	1	82	1	358	1					
1	107	0.856	0.741	34	0.432	0.260	118	2.008	0.070	242	0.833	0.457		
<b>Housing construction</b>														
Wood	68	1	2	1	1	26	1	69	1					
Concrete/Stone/Bricks	132	0.596	0.274	198	0.884	0.608	174	0.780	0.622	531	1.457	0.365		
<b>Window number</b>														
0-9	127	1	81	1	1	142	1	357	1					
≥ 10	73	0.930	0.883	119	0.871	0.757	58	1.111	0.786	233	2.252	0.001		
<b>Having backyard</b>														
No	62	1	75	1	1	41	1	194	1					
Yes	138	0.817	0.683	125	1.142	0.775	159	1.958	0.185	406	1.457	0.161		

A highlighted significant OR (P < 0.05) and/or OR greater than 1.5 times was considered a risk.

\*Significant predictors further used for multivariate analysis as shown in Table 28

Determinants significantly associated with dengue transmission in univariate analyses of the ecotope of RCDENPURA (which was previously considered as risk ecotope) were selected for the final multivariate regression. These significant variables were: sex, movement during the last 3 months, years of schooling, average family income, comprehensive knowledge about dengue, application of mosquito repellent, house floor number, and house with a backyard. In multivariate analysis, the interactions between these selected determinants and their confounding were tested—the results of which are presented in Table 28. There were only 2 factors observed to remain a significant predictor for dengue transmission. The two strongest predictors were history of movement during the last 3 months and the respondents' years of schooling (OR=1.874, 95%CI=1.056-3.325 and OR=1.544, 95%CI=1.061-2.245). People who had a history of movement during the last 3 months, as well as persons who had completed  $\geq 5$  years of schooling, were found to have a consistent and strong association with dengue transmission.

**Table 28** Multivariate analysis of significant socio-demographic determinants for dengue transmission in the ecotope of residential mixed with commercial area and DENPURA.

Socio-demographic significant determinants	% dengue case history	OR	95% CI
Sex			
Male	10.4	1	
Female	24.1	1.434	0.987-2.084
Movement during last 3 months			
No	18.5	1	
Yes	31.3	1.874	1.056-3.325
Years of schooling			
0-4	15.2	1	
≥ 5	22.3	1.544	1.061-2.245
Average family income (Baht)			
≥ 4500	14.4	1	
≤ 4499	23.6	1.416	0.994-2.016
Comprehensive knowledge about dengue			
High	29.6	1	
Low	12.6	1.361	0.847-2.185
Applying mosquito repellent			
No	22.0	1	
Yes	3.7	1.192	0.731-1.943
House floor number			
≥ 2	14.5	1	
1	12.4	1.007	0.715-1.418
House with a backyard			
No	12.2	1	
Yes	21.4	1.436	0.982-2.101

## CHAPTER V

### DISCUSSION

The ultimate goal of this research was to gain an insight into the eco-bio-social factors that were hypothesized to play a pivotal role in dengue transmission dynamics and persistence in urban and semi-urban settings. The research outcomes originating from empirically significant findings were expected to help in identifying appropriate control strategies and better management solutions for diverse ecotopes embedded in an urban ecosystem. The study fully integrated multiple disciplines to determine what underlying drivers greatly contribute to the persistence of dengue in an urban environment. And, as far as I know, it is the first one to characterize which ecotope significantly affects *Aedes* vector density and poses the most risk for dengue occurrence. In addition, this report marks the first time that an application of mosquito blood meal analysis has been used to assess dengue herd immunity in urban and semi-urban localities in Thailand.

Accordingly, this thesis chapter provides a series of meaningful scientific findings in relation to research questions, along with sequential-sectioned discussions and suggestions.

#### 5.1 Cluster Characteristics

Buffered against environmental intimacies by civilization and technological development, the human population is vitally dependent upon the cycling of 4 service components of an ecosystem: supporting, provisional, regulating, and cultural (Corvalan, Hales, McMichael, Millennium Ecosystem Assessment (Program), & World Health Organization., 2005). Since all levels of a human ecosystem are a result of interactions among humans, natural constituents, and processes (Ellis & Wilcox, 2009), the emergence of dengue reflects a causal relationship between the human host, the dengue virus pathogen, and *Aedes* vector, shaped by environment (Guzman & Kouri, 2002; Halstead, 2008; Rigau-Perez et al., 1998).

In this study, a cluster background survey was undertaken in all 12 study clusters by using a cluster background survey form. Three major dimensions of cluster characteristics were carefully examined, including socio-demographics, basic infrastructure, and geographical features.

In general, all of these features varied among the entire 12 study clusters, except for basic electrical infrastructure. For the socio-demographic dimension, the 6 urban clusters were likely to have higher socio-economic status (SES) and better housing conditions than those 6 clusters in the semi-urban area. More precisely, the SES of the 6 urban clusters ranged from the lowest to the highest classes, while the latter semi-urban group did not differ from each other. The SES of the commercial ecotope was greater than any other ecotope in the study area, while that of DENPURA ecotope was observed to be lowest. Moreover, among 4 ecotopes, the housing structures and conditions were also observed to have heterogeneity in their compounds and complexes. The commercial ecotope area mostly contained well-equipped 2- to 3-floor houses in good condition. The RCDENPURA and RC ecotopes had 1- to 2-storey households in fair condition. The DENPURA ecotope had the poorest sanitation in its 1- to 2-floor houses. These socio-environmental conditions reflect the sequential outcomes of civil planning and development, especially urbanization, both within the different ecotopes and between the cities and the semi-urban areas.

The basic infrastructural services investigated in the study areas were: electricity, water supply service, and garbage management. All houses in the study site had full access to electricity, even households located quite far from the main road. A majority of households situated in either urban or semi-urban vicinities received a public water supply and garbage management services from local municipalities, waterworks authorities, and/or private companies. Only the DENPURA ecotope with a low degree of dengue transmission seemed to have irregular garbage collection and lacked a public water supply service. Consequently, inappropriate waste disposal management among householders and poor water drainage which left polluted stagnant water under elevated dwellings created a great number of *Culex* breeding places instead of suitable oviposition sites for dengue vector populations. The lack of a piped water supply did not limit these inhabitants' water usage, however, because most dwellers used canal water and their own pump system. Despite nearly all

households having access to public water supply systems, many inhabitants found the usage of piped water unpleasant due to frequent turbid water and supply interruptions.

When the geographical characteristics were taken into consideration for individual clusters in terms of the density and distribution of households (as well as public spaces and green areas), the proximity of households in the urban clusters was closer than that observed in the semi-urban clusters. This indicated that there has been exploitation of land use, along with higher settlement, in the urban localities. Meanwhile, the DENPURA and commercial areas appeared to exhibit the greatest concentration of buildings and/or public spaces among the four ecotopes; the more dense households and public spaces, the fewer green areas. More distant household distribution was commonly found in the other two ecotopes, RCDENPURA and RC.

The socio-demographic, basic infrastructure, and geographic characteristics of the typical 12 clusters presented in this section provided a basic understanding of the cluster background for conceptualizing some vulnerability of *Aedes* mosquito abundance and dengue transmission, as well as the prerequisite information for further investigating the eco-bio-social determinants underlying vector density and dengue emergence. In fact, many relevant studies have demonstrated that it is this set of micro-socioeconomic, infrastructural, and environmental parameters embedded in communities which appears responsible for either increased numbers of *Aedes* mosquitoes or increased epidemic transmission of the dengue virus in particular ecosystem localities (Aagaard-Hansen et al., 2009; Brightmer & Fantato, 1998; Caprara et al., 2009; Galli & Chiaravalloti Neto, 2008; Gleiser & Zalazar, 2009; Holling, 2001).

## **5.2 Ecological Investigations**

Dengue has been primarily a mosquito-borne disease found in urban and semi-urban settings. The morbidity and mortality of this disease may vary significantly from one place to another because of different local parameters. The dengue problem posed by an expansion in space and time is compounded by three major eco-bio-social drivers. Understanding these factors that cause dengue vector increase and/or expand their geographical range (and thereby the disease) is essential to designing interventions that minimize negative impacts and prevent disease epidemics from

occurring. Such an ecological perspective can provide foresight into the appropriateness of dengue control efforts, give answers to unexpected vector control responses, and contribute to effective management solutions in an ever-changing environment.

For this study, a novel ecosystem concept was created to exhaustively scrutinize the associations between ecotopes and *Aedes* vector abundance and the transmission of dengue fever in the study areas. In addition to ecotope as an ecological predictor, the degree of urbanization (urban versus semi-urban), as well as the intensity of dengue transmission, were other determinants used to assess risk for *Aedes* mosquito increases and dengue transmission. Interestingly, differences between urban and semi-urban areas did not pose risks for higher vector abundance or dengue transmission. One possible explanation for this is that some common characteristics (such as socio-demographic and biological factors) may coexist between two similar settings. Settings with a high degree of dengue transmission corroborated the risk for the transmission of dengue, but not for an increase in dengue vector density.

Along the same lines, painstaking investigations of the ecological effects of diverse ecotopes on increases in *Aedes* population intensity and dengue occurrence have indicated that those typical individual ecotopes exhibit different risks for the two dependent variables. Of all 4 ecotopes, only the ecotope of RCDENPURA exhibited a strong association with dengue transmission risk, suggesting that the complexity of the urban ecosystem (where the ecotope is in fact a combination of the R, C, and DENPURA ecotopes) may give rise to dengue emergence. Furthermore, higher significant association between this ecotope and dengue transmission was demonstrated when individual ecotopes in high dengue transmission areas were applied. However, none of the four ecotopes was significantly associated with the increase in pupal demographic index. This suggested that the typical ecotopes themselves may not directly affect *Aedes* mosquito density. On the other hand, an analysis of the association between individual ecotopes in high dengue transmission localities and vector density revealed that the two ecotopes of RCDEPURA and RC were a significant predictor for *Aedes* vector abundance.

In fact, there is significant evidence, in particular, regarding the relationship between ecological factors and mosquito population density and/or

dengue transmission (Almeida et al., 2005; Chakravarti & Kumaria, 2005; Ellis & Wilcox, 2009; Koopman & Longini, 1994; Laird, 1963; Wearing & Rohani, 2006). The relevant ecological determinants that have proved significantly favorable for either of the two outcome variables (vector and disease) are change in land use, urbanization, and natural habitat alteration, driven by human population growth and economic expansion. Additionally, signal increases in the rate and extent of environmental degradation over the last century, largely resulting from human activities, have fueled a growing number of artificial water-bearing containers that harbor *Aedes* mosquitoes, increasing dengue case expansion in both space and time.

### **5.3 Biological Implications**

This section studies the biological implications of the *Aedes* mosquito vector, as well as dengue herd immunity among human populations in relation to dengue transmission dynamics. The first part discusses entomological findings in terms of container types, together with their social uses and productivity. The second part debates the novel application of mosquito blood meal analysis for determining dengue herd immunity in human populations.

#### **5.3.1 Evaluation of *Aedes* vector abundance and relevant environmental factors in the wet and dry seasons**

In the absence of an effective dengue vaccine, control of *Aedes* populations via source reduction is still considered the most effective way to prevent and control dengue, although it has proven ineffective or unsustainable in many areas with a history of mosquito control problems. Urban areas in a tropical zone may favor dengue viral transmission to humans through close contacts between the *Aedes* vectors and the human hosts; and they also may positively affect mosquito populations by offering larval habitats, refuges, and an adequate microclimate to survive the cold season. The urban ecosystem in different geographical areas constitutes diverse landscape characteristics. The impact of environmental factors on prevalence of *Aedes* mosquitoes along with the effect of *Aedes* vector abundance on dengue virus transmission have been the subject of much recent empirical and analytical research.

The main purpose of this section is to elucidate and debate the research findings obtained from entomological surveyed data of wet and dry seasons in relation to water container types, their social use, and productivity.

For the wet season survey, at least one water-filled container was observed in 1,194 of 1,200 houses inspected. The 7,804 water containers were categorized into 11 categories: drum, cement bath basin, jar (50-400L), bucket, circle cement tank, lotus bowl, flower vase, used tire, plastic container, small local jar (<50L), and other containers. The 50-400L jar that constituted a half of all container types was found to be used most frequently during this season, followed by the cement bath basin at about 14%. The association of container characteristics with their social use and ecotope types was statistically tested. The two most frequent water container types (50-400L jar and cement bath basin) were observed in the 3 ecotopes of RCDENPURA, DENPURA, and RC, whereas the four most frequent container types in the C ecotope were: cement bath basin, jar (50-400L), bucket, and lotus bowl.

Regarding water type, most containers held tap or well water in all ecotopes, while containers holding rainwater or canal water constituted about 10-40%. This suggested that all ecotopes relied mainly on tap/well water. Although there was a statistical difference between some container characteristics (under vegetation, location, shade) and the four different ecotopes, a majority of the containers from every ecotope were located far from vegetation, inside the house, and in full shade. Concerning social use, research findings revealed that most of the containers were used during the past 7 days, and half had been treated to prevent mosquito breeding and had a cover. These findings suggested that nearly a half of the water-bearing containers possessed conditions favorable for *Aedes* breeding.

Further investigations included the analysis of water-holding container characteristics and their social use in terms of productivity in relation to the 4 diverse ecotopes. The relationship between container characteristics and their social use and the number of immature-positive containers varied among the ecotopes. However these findings were consistent with previous studies (Chansang, 2005; Chareonviriyaphap et al., 2003; Kittayapong & Strickman, 1993; Strickman & Kittayapong, 2003) that showed that 50-400L jars and cement bath basins to be the main breeding sources or key containers and candidates for improved targeted

interventions in all four examined ecotopes. Furthermore, results of associations among container characteristics and their social use showed that containers holding tap/well water, located far from vegetation, inside the house, in a fully shaded area, and unlidged were all more likely to be infested by *Aedes* larvae and pupae. Research results of entomological indices demonstrated the differences among the ecotopes. The 3 traditional indices of HI, CI, and BI varied among ecotopes, while the DENPURA and RC ecotopes showed the highest 0.47 and 0.42 pupae per person, respectively. These three traditional indices have been deemed ineffective in predicting dengue transmission and consequently are no longer used for critical surveillance. Pupae per person is considered most valid and gets used for assessing risk and directing control operations. These findings indicated the two ecotopes mentioned above posed the higher risk of dengue transmission, compared to those of RCDENPUA and C.

For the dry season survey, at least one water-filled container was observed in 1,193 of 1,200 houses inspected. The 6,836 potential containers were categorized into 11 groups: drum, cement bath basin, jar (50-400L), bucket, circle cement tank, lotus bowl, flower vase, used tire, plastic container, small local jar (<50L), and other containers. As observed in the wet season, the 50-400L jar that constituted half of all container types was the most frequent container used during the dry season, followed by the cement bath basin, which accounted for about 16%. The association of container characteristics along with their social use and ecotope type was statistically tested. The two most frequent water container types (50-400L jar and cement bath basin) were observed in the RCDENPURA, DENPURA, and RC ecotopes, whereas the C ecotope revealed the four most frequent container types: cement bath basin, jar (50-400L), bucket, and lotus bowl. This observation indicated that local inhabitants in the commercial ecotope may have had additional lotus bowls which they used for recreation and decorating the house. Regarding water type, most containers held tap or well water in all ecotopes, while containers holding rainwater or canal water constituted about 10-40%. This suggested that all ecotopes relied mainly on tap/well water. Although there was a statistical difference between some container characteristics (under vegetation, location, shade) and the four different ecotopes, a majority of the containers from every ecotope were located far from vegetation, inside the house, and fully shaded. Regarding social use, research findings revealed

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### **5.3.2 The application of mosquito blood meal analysis to assess dengue herd immunity in human populations in the wet and dry seasons**

Information regarding anti-dengue human herd immunity and spatio-temporal distribution of dengue viruses in human populations relies primarily on the detection of antibodies specific for 4 DENV antigens by enzyme linked immunosorbent assays (ELISA). In general, a dengue surveillance that involves the application of human blood serological surveys has to follow safety and ethical rules; hence this slows down

the process and potential of rapid risk assessments. The ELISA approach of using *Culex* mosquitoes that have engorged themselves on humans proves a useful method for the effective detection of anti-DENV antibodies (Barbazan et al., 2009). The principal aim of this part is to discuss the development and application of mosquito blood meal analysis to serologically determine positive rates of dengue herd immunity in human populations, as well as to temporally and spatially analyze the distribution of these dengue herd immunity backgrounds.

During the wet season, a total of 715 blood-fed *Culex* mosquitoes were examined for human IgG by using ELISA. Among all *Culex* species identified, *Cx. quinquefasciatus* was observed the most, confirming that this species is most predominant and is widely distributed in the residential areas of the study clusters and elsewhere in Thailand. However, human IgG seropositive rates did not significantly differ among all blood-engorged *Culex* species. This suggests that any *Culex* species can be used to determine human IgG present in mosquito blood meals. Furthermore, half of the *Culex* blood meal samples were found to contain human IgG, indicating that these Culicine mosquitoes can bite a variety of hosts and that their habitats are situated within and around human habitations. It was striking to note that human IgG-seropositive rates obtained from high and low dengue transmission areas were not significantly different. Nonetheless, the rates of human IgG seropositivity were about twice as high in urban areas, compared to semi-urban communities. These findings imply that *Culex* abundance and distribution may be regulated by human population density or urbanization.

With respect to the determination of human anti-DENV IgM and IgG antibodies, the human IgG blood meal samples gave seropositive identifications of 17% DENV IgM and 10% DENV IgG. The seropositive rates of DENV IgM and IgG in a high dengue transmission setting was significantly greater than in a low dengue transmission area, showing a strong relationship between the dengue herd immunity background and the endemicity of dengue transmission. More precisely, the high dengue transmission settings used in this study reflected the anti-dengue virus immune status of the local populations in the studied areas, where high numbers of dengue cases had been reported for consecutive years. These findings agreed well with several previous studies, where the serological survey of anti-DENV IgM and IgG herd

immunity in a given population has potentially been as applicable as the epidemiological approach when exploring dengue endemicity and predicting risk for dengue transmission (Anantapreecha et al., 2005; Brown, Vickers, Salas, & Smikle, 2009; Hati, 2009; Peyerl-Hoffmann et al., 2004; Rahman et al., 2007). Thus, this study appears to have proposed a promising method for dengue surveillance.

The seroprevalence of anti-DENV IgM and IgG in an urban community did not differ from those in a semi-urban locality. When the seroprevalence was further analyzed for the four different ecotopes across the 12 clusters, DENPURA with a high degree of dengue transmission showed the highest DENV IgM seropositivity rate, while the highest measures for DENV IgG seropositivity were seen in RCDENPURA with a low degree of dengue transmission, DENPURA with a high degree of dengue transmission, and RC with a high degree of dengue transmission. In fact, anti-DENV IgM seroprevalence accounts for active dengue transmission; in other words, it generalizes current dengue virus circulation in the serologically examined population, while anti-DENV IgG seroprevalence indicates past history of dengue transmission in the affected local community. In such findings, it is not unusual to see high levels of anti-DENV IgM and IgG seroprevalences in the 3 high dengue transmission ecotopes. But in the case of RCDENPURA with a low degree of dengue transmission, the seroprevalence of anti-DENV IgG was high. This apparent paradox can be explained by the fact that there are some other factors involved, including an insufficient number of representative mosquito population specimens and a sampling bias resulting from an abnormal distribution of the *Culex* mosquitoes obtained.

In addition, the differences in seroprevalence for anti-DENV IgM and IgG were tested for all 6-pair clusters with high and low degrees of dengue transmission. For the anti-DENV IgM, only the DENPURA ecotope with a high degree of dengue transmission had a seroprevalence 4-fold higher than that seen in an ecotope with a low level of dengue transmission. As for assessing anti-DENV IgG, the DENPURA and RC ecotopes, with high and low degrees of dengue transmission, were also found to have significant differences in their seropositivity rates. These findings suggest that past and active dengue occurrence had taken place in DENPURA with a high degree

of dengue transmission, while RC with a high degree of dengue transmission reflected only past dengue infections that had developed over a few years in this area.

Ecotope characteristics as risk factors for past and active dengue occurrence were also examined. Urbanization was not associated with past or active dengue occurrences. Regarding degree of dengue transmission, the high transmission ecotope posed a significant risk for active and past dengue occurrences, as well as a combination of the two. More interestingly, the same high transmission ecotope doubled the risk for a combination of past and active dengue occurrence. Associations between ecotope type and dengue occurrence were also made. DENPURA was observed to be a significant predictor for active dengue transmission, as well as for a combination of past and active dengue transmission, while RCDENPURA and RC had a significant risk only for a combination of past and active dengue transmission.

Followed by an entomological survey in the wet season, the same 1,200 households were repeatedly examined for adult mosquito collection in the dry season. Results from the *Culex* species identification of the 760 blood-fed *Culex* mosquitoes that were obtained showed that *Cx. quinquefasciatus* produced a major portion of the total. This observation corroborated well with the data observed earlier during the wet season, when a great abundance of *Cx. quinquefasciatus* had been commonly observed near and around the residential zones of the study clusters and elsewhere in Thailand. Among all blood-engorged *Culex* species used for the determination of human IgG, it was mostly *Cx. sitiens* and *Cx. quinquefasciatus* which were observed to contain human blood. Similar to findings in the earlier season, the human IgG seropositive rates did not reveal a significant difference among the *Culex* species evaluated; this increases the possibility that any *Culex* species is a good proxy measure for the determination of human IgG present in mosquito blood meals. During this season, about one third of the *Culex* blood meal samples were found to contain human IgG, which was less than those observed in the wet season (approx. 50%). The available blood sources for these Culicine mosquitoes may vary between the two seasons and thus serve as an explanation for these findings.

In contrast to the human IgG seropositivity rates derived from the wet season, the rates of human IgG seropositivity in this season were nearly twice as high in semi-urban areas than those in urban communities. Subsequently, all human IgG

seropositive samples were used to determine DENV IgM and IgG, with the results being 38% DENV IgM and 36% DENV IgG. The seropositive rates of DENV IgM and IgG in high dengue transmission settings were significantly greater than in low dengue transmission areas, indicating a strong relationship between the dengue herd immunity background and the endemicity of dengue transmission. More precisely, the high dengue transmission settings used in this study reflected the anti-dengue virus immune status of the local populations in the studied areas, where large numbers of dengue cases might have continuously developed. These findings agree well with the observations of this study during the wet season and prior studies, in that the serological survey of anti-DENV IgM and IgG herd immunity in a given population can potentially serve as the epidemiological approach for exploring dengue endemicity and predicting risk of dengue transmission.

The seropositivity rates of anti-DENV IgM and IgG in an urban neighborhood were different from a semi-urban locality. When these seropositivity rates were further analyzed to see the distribution among the 12 clusters, the DENPURA ecotope with a high degree of dengue transmission demonstrated the highest DENV IgM seropositivity rate, whereas the 3 highest measures for DENV IgG seropositivity were observed in the DENPURA with a high degree of dengue transmission, the RCDENPURA with a high degree of dengue transmission, and the RC with a high degree of dengue transmission. These research results contribute to a better understanding of dengue transmission dynamics in light of ecotope characteristics and different degrees of dengue transmission.

The seropositivity rates were distinctly compared among the same ecotope to look for differences between the degrees of dengue transmission within the different ecotopes. As to anti-DENV IgM, a significant difference in seropositivity rate was observed only in the DENPURA ecotope paired with high and low degrees of dengue transmission. For anti-DENV IgG, only one pair of commercial ecotopes with high and low degrees of dengue transmission statistically differed in seropositivity rate. Considering these findings, the DENPURA ecotope that showed a high dengue seroprevalence in relation to seasonal variation must have maintained dengue virus circulation for several years up to the present study between the wet and dry seasons,

while the high degree of dengue transmission in the commercial ecotope might only be a reflection of the development of past dengue infection from previous years.

As in the wet season, ecotope characteristics as risk factors for past and active dengue occurrence were examined. Urbanization was not associated with either past or active dengue occurrences. Regarding the degree of dengue transmission, the high transmission ecotope posed a significant risk for active and past dengue occurrences, as well as in combination. Lastly, associations between ecotope type and dengue occurrence were made. Only DENPURA was observed to be a significant predictor for past dengue transmission.

Results of seasonal and spatial analysis of anti-dengue herd immunity in relation to reported dengue cases will now be discussed. Overall, the seroprevalence rates of both anti-DENV IgM and IgG antibodies observed in the dry season were significantly higher than those seen in the wet season. For anti-DENV IgM, an analysis of seroprevalence of individual clusters was undertaken to compare the discrepancy associated with seasonal variation. Of all 12 clusters examined, three clusters (numbers 1, 4, and 7) were found to significantly exhibit higher seropositivity in the dry season, compared to that in the wet season. As to anti-DENV IgG, five clusters (numbers 1, 3, 4, 7, and 9) were observed to have similar seropositivity in the dry season, which was significantly higher than that observed in the wet season. These high DENV IgM and IgG seropositivity rates in the dry season were also associated with urbanization, degree of dengue transmission, and type of ecotope, except for DENPURA. These findings do not correspond to actual dengue case numbers reported during the same period of time of any season. Indeed, dengue incidence in Thailand exhibits a strong seasonal pattern in which transmission normally occurs during the rainy season. The unique occasions which we found can be explained in light of reproductive number ( $R_0$ ) and human population immunity. That is, the high DENV IgM and IgG seroprevalence observed in the dry season might have been a result of dengue viral circulation activities emerging below the transmission threshold level among asymptomatic cases in receptive communities.

Finally, the DENV IgM and IgG seropositivity rates taken from mosquito blood meal analysis in individual clusters during two different seasons were correlated cluster by cluster with actual dengue case numbers reported during the same period of

time. It was found that nearly all of the clusters demonstrated an association between DENV seropositivity rate and numbers of reported dengue cases. In particular, the DENV IgM seropositivity rates for the ecotope of DENPURA with high and low degrees of dengue transmission correlated well with dengue cases reported in both the wet and dry seasons. This strong correlation was also found for DENV IgG seropositivity rates in the DENPURA and RC ecotopes during the wet season, as well as the commercial ecotope in the dry season. These findings confirm that mosquito blood meal analysis could be used as an alternative approach to survey seroprevalence and assess herd immunity for dengue fever in human populations.

## **5.4 Social Science Research**

### **5.4.1 Examination of socio-demographic parameters underlying dengue transmission**

Socio-demographic factors are commonly targeted for disease prevention and control and underpin successful public health programs. Although there have been promising indications in the literature, these parameters have remained poorly understood in the case of dengue.

The main objective of this section is to thoroughly examine and statistically compare socio-demographic factors between two different areas with high and low degrees of dengue transmission. The studied factors included: the general characteristics of household members and their past history of dengue infection; housing and basic services; knowledge about dengue; and vector-related knowledge and practices. In the beginning, all background characteristics of 1,200 heads of household and 1,200 respondents in high and low dengue transmission areas were analyzed by proportion testing. The factors of gender, age group, religion, years of schooling, highest school degree attained, and occupation statistically differed between the two areas. These research results were further used to examine several other factors of interest underlying the transmission of dengue. In doing so, baseline information and previous history of dengue infection of 4,166 household members were compared to see whether there was a significant difference between the two settings. Research results revealed that living in a high dengue transmission area was strongly related to previous history of dengue infection. This implied dengue

prevalence and epidemic stability in the areas with a high degree of dengue transmission. Regarding housing and basic services of households in high and low dengue transmission localities, it was revealed that house components of concrete/stone/brick and factors related to the management of solid waste and rubbish disposal facilitated conditions favorable to dengue transmission. Similar results were also recorded during the evaluation of house structures and surroundings (Brightmer & Fantato, 1998; Nakhapakorn & Tripathi, 2005; Rudnick, 1986; Thammapalo, Chongsuvivatwong, Geater, & Dueravee, 2008).

To see whether there was an association related to dengue knowledge among the respondents living in the two settings, all respective knowledge features were individually examined. Overall, dengue knowledge of individuals in the low dengue transmission setting was significantly higher than that in the high dengue transmission setting. The vector-related knowledge of the respondents appeared to be, in similar fashion, associated with the degree of dengue transmission. These results are in keeping with the health belief model, which says that people who have sufficient knowledge and/or high attitude are likely to exhibit appropriate behavior. Health belief and behavioral change models have been used routinely to develop materials to promote community participation in the prevention and control of dengue (Lennon, 2005). In examining dengue information sources, the only obvious difference between the two areas was related to the second source of dengue information. Speaking specifically, hospitals and health workers were the two most common information sources for the two settings. However, neighborhood was a significant source in the high dengue transmission area, whereas radio played an important role in the dissemination of dengue information in the lower endemic area. These observations suggest that high dengue transmission areas, where dengue epidemics normally take place, may have much more local communication about dengue via members of the neighborhood, while mass media organizations such as radio stations might regularly deliver dengue news in low dengue transmission areas. Nevertheless, numerous studies have demonstrated mass media like radio and television are main sources of dengue information for local people (Haldar et al., 2008; Itrat et al., 2008; Naeem-Ullah & Akram, 2009; Nalongsack et al., 2009; Phuanukoonnon et al., 2006; Phuong

et al., 2008). Such findings are useful for selecting the appropriate channel to deliver dengue messages to the respective areas.

As to vector control activities by the household and government, our research findings suggest that control efforts consisting of some indoor spraying and fogging were implemented after a disease outbreak, while the measures used to prevent disease emergence included putting mosquito-eating fishes in water containers, checking water containers, and adding drugs and chemicals to water containers.

Ultimately, the association between socio-demographic parameters and dengue transmission deserves further study, and may vary according to the settings of each urban and semi-urban ecotope. For this study, it is likely that the emergence of dengue is associated with non-socio-demographic factors.

#### **5.4.2 Stakeholder analysis**

Having completely examined the socio-demographic factors, together with the ecological and biological determinants of dengue infection, stakeholder analysis in relation to function and interest was undertaken to triangulate with the household and dengue vector survey data and ecotope assessments in terms of contextualized eco-bio-social system analysis. According to the checklist method, all 32 interviewed stakeholders were identified based on 3 criteria: type, organization, and, mission. A majority were government public health agencies, which suggests that the dengue problem in this region is foremost the burden of public health organizations, despite the fact that other respective institutions should have joined and participated in the dengue prevention and control programs in which they have been involved. After the identification process, all stakeholders were successively profiled by applying the Social Analysis CLIP. Out of the seven profile categories, 20 stakeholders were characterized as “dominant”, while 6 were identified as “marginalized”. Three others were profiled as “forceful”, and three more “influential”, while none were labeled as “vulnerable”, “dormant”, or “respected”. These observations suggest that actions must be taken to empower the marginalized groups that have pressing needs or interests.

These 32 stakeholders were then regrouped based on their mission, the majority being responsible for public health services. An analysis of existing

collaborations and conflicts between the provincial public health office (PPHO) and other 31 stakeholders suggested that almost all public health stakeholders had a two-way relationship, while others responsible for garbage management, water supply, and urban planning appeared to possess one way relationship from PPHO to them. Remarkably, the public health district and health center offices had a strong relationship with the PPHO. This robust association may be due to the line of command between them. No negative influences were observed in any pair of stakeholders, suggesting that conflicts of interest may not commonly occur among respective stakeholders involved in the dengue and/or *Aedes* mosquito control problems within the study area. However, the PPHO needs to work toward building a strong two-way relationship with some stakeholders (particularly those which are mainly responsible for garbage management, water supply, and urban planning).

Lastly, a contextualized determination of the eco-bio-social-system was concluded with regards to the forces that contribute to dengue persistence in urban and semi-urban areas. In summary, the research results provided various lines of empirical information regarding dengue, as well as evidence useful for the design of dengue prevention and control programs, particularly in urban ecosystem localities. Like the choice of study areas, the ecotope concept used in this study is capable of being applied to urban and/or semi-urban settings elsewhere. For example, it was found that RCDENPURA ecotope, which had been clearly considered most at risk for dengue transmission, should be focused on first when implementing public health and/or dengue control programs, especially in situations where there are low budgets or insufficient public health manpower. Another important finding was that human movement and the educational background are the two greatest determinants observed to play a role in the occurrence of dengue in such ecotope as RCDENPURA. This useful information can be applied in developing effective dengue prevention and control programs and can be used as the baseline data for further analysis in light of socio-demographic factors. In addition, among the 4 individual ecotopes, entomological and immunological findings obtained from particular areas can affordably be applied to respective ecotopes in combating urban dengue.

Findings from this study suggest that combating and pursuing urban diseases like dengue in efficient and sustainable ways need not only take into

consideration the underlying biotic and abiotic determinants of the disease but also the respective stakeholders and their environment. An understanding of the strengths and weaknesses of respective stakeholders involved in dengue and/or vector control problem(s) in a particular ecotope can be used to develop strategies to empower responsible stakeholder teams towards implementing effective solutions to dengue problems. In designing any effective sustainable dengue control intervention for endemic urban areas such as Chachoengsao Province, considerations should go beyond the health belief model and routine classical vector control methods to focus on novel comprehensive approaches which are compatible with the socio-demographic bio-ecological backgrounds of the affected area.

### **5.5 A Comprehensive Integrated Investigation of Ecological and Socio-demographic Determinants as Epidemiological Risk Factors for Dengue**

Risk factors related to dengue transmission are very much influenced by individual and environmental determinants. Employing a univariate logistic regression analysis, significant socio-demographic risk factors as vulnerable characteristics for dengue transmission were elicited from 1,200 respondents living among 4 respective ecotopes in the study area. For the DENPURA ecotope, the significant socio-demographic factors associated with past history of dengue infection among the respondents included: female sex; movement during the last 3 months; average family income lower than 4,500 baht per month; years of stay in house longer than 10 years; poor knowledge about oviposition sites of *Aedes* mosquitoes; low/fair attitude about gender roles and not serious/low attitude about the seriousness of dengue; household practices relating to the regularity of emptying medium-size water containers and fogging against mosquitoes; community effort to clean the environment; and house attachments. For the commercial ecotope, the significant parameters in predicting dengue transmission included: female sex; unemployment; movement during last 3 months; average family income lower than 4,500 baht per month; poor knowledge regarding dengue spread and low overall dengue knowledge; low attitude regarding family roles and government roles; household practices relating to the storage of water

and the regularity of emptying small-size water containers; community effort to clean the environment; and residential purpose of housing.

For the ecotope of RCDENPURA, research results revealed some significant risk factors for dengue transmission. These pronounced predictors included: female sex; movement during the last 3 months; years of schooling longer than 5 years; average family income lower than 4,500 baht per month; poor knowledge regarding dengue spread and low overall dengue knowledge; low attitudes about family and government roles; household practices relating to the regularity of emptying medium- and large-size water containers; the application of repellent to reduce mosquito nuisance and fogging against mosquitoes; houses with one floor; and houses with a backyard.

For the RC ecotope, the factors associated with a high risk for dengue fever included: years of schooling longer than 5 years; not serious/low attitude about seriousness of dengue; household practices related to the regularity of emptying large-size water containers; and houses having  $\geq 10$  windows.

In conclusion, the associated socio-demographic variables considered as significant predictors for dengue transmission in the study area that covered 4 various ecotopes (encompassing urban and semi-urban areas) included the following: sex; occupation; movement during the last 3 months; years of schooling; average family income; comprehensive and overall dengue knowledge regarding dengue spread and *Aedes* breeding places; attitudes about gender/family/government roles and seriousness of dengue; household practices relating to the storage of water; the regularity of emptying large/medium/small size water containers; the application of mosquito repellent and fogging; community effort to clean the environment; house attachments; main purpose of housing; house floor number; and house having a backyard.

Overall, significant predictors for recent dengue infection slightly differed among the 4 ecotopes. However, the findings show that some socio-demographic factors likely play a common role in dengue transmission for more than one ecotope. Understanding male-female differences in infection rates and the severity of the disease is essential for public health control activities. For this study, the 3 ecotopes of DENPURA, C, and RCDENPURA reported twice the number of females experiencing

dengue compared to males. This observation however is not consistent with the majority of earlier findings that have demonstrated a greater risk for dengue infection in male than female (Guha-Sapir & Schimmer, 2005; Rodriguez-Figueroa, Rigau-Perez, Suarez, & Reiter, 1995; Vanwambeke et al., 2006); however, a study conducted in Maxio in 1980 demonstrated a higher proportion of woman among the surveyed samples was at risk of dengue infection (Kaplan et al., 1983). This incidence bias in favor of females may have resulted from the timing of survey interviews, which was in the daytime when more women are generally at home.

As to occupation, only the commercial ecotope showed unemployed persons with the highest risk for dengue infection compared to skilled and unskilled workers. Because unemployed persons usually spend most of their time at home, this could contribute to them being more likely bitten by dengue-infected *Aedes* mosquitoes, which mostly bite during the daytime. Interestingly, movement of respondents during the last 3 months was found to be a significant predictor in the three ecotopes in the urban area, which is where a variety of jobs are largely provided. In the RCDENPURA and RC ecotopes, persons with longer years of schooling had a greater risk of dengue infection than those with a lower education, suggesting that there is a link between years of schooling and movement that further increases the risk of dengue infection.

With regards to average family income (AFI), the 3 ecotopes in an urban setting showed that an AFI lower than 4,500 baht increased risk of dengue infection compared to one higher than 4,500 baht. Additionally, family members who stayed at home more than 10 years was confirmed as a significant predictor for dengue occurrence, but only for the ecotope of DEPURA.

A number of potential risk factors associated with the respondents' past history of dengue were elucidated during this study. Despite their importance in developing effective strategies for behavior change, knowledge, attitude, and practice (KAP) studies are rare; hence little is known regarding the knowledge and attitude of the exposed population towards dengue. This cross-sectional inquiry among 1,200 participants in all different ecotopes showed that poor dengue knowledge and poor to fair attitudes regarding gender/family/government roles and the seriousness of dengue

and mosquito control were significant risk factors that greatly contributed to the occurrence of dengue in the studied areas.

As for household practices related to vector control, the practice of storing water posed the greatest risk for dengue transmission only in the commercial area ecotope. Actually, every surveyed house in this ecotope fully relied on a piped water supply, yet still stored water for domestic use. This practice might have been due to the irregular distribution of piped water (as a result of broken water pipes) and/or the poor quality of the piped water caused by colloidal suspension. Irregular emptying of the water container was observed to be a significant risk factor for dengue transmission, a risk that varied in relation to the size of the containers and the ecotopes. These research results suggest that potential *Aedes* breeding containers among typical ecotopes should be targeted for vector control efforts and public health programs. In contrast, this research found that some practices commonly used to reduce mosquito nuisance, including applying repellent and fogging, are inversely associated with dengue transmission. This contradiction might be explained by the fact that these vector control practices could have been applied as post-epidemic control measures.

Housing and basic services were the other socio-demographic predictors statistically tested. House attachments created the right condition for dengue transmission in only the ecotope of the densely populated residential area, suggesting that houses with residential purpose were likely to create more artificial breeding habitat for the *Aedes* vector than those with business and/or restaurant purposes. Last but not least, three housing characteristics (house with only one floor, house with more than 10 windows, and house having a backyard) significantly contributed to dengue transmission in only the RCDENPURA and RC ecotopes. These results suggest that characteristics of housing structure and basic services in residential areas mixed with commercial (or commercial and DENPURA) contributed significantly to a close contact between household members and *Aedes* female mosquitoes, thereby increasing the risk of dengue infection.

Examining dengue risk factors through the lens of a multivariate logistic regression model, the research results showed that only 2 out of the 8 previously-considered significant variables were found to maintain their risks for dengue

emergence in RCDENPURA, the most at risk ecotope. These two strongest predictors included movement during the last 3 months and years of schooling longer than 5 years. The finding that human movement significantly favors the transmission of dengue corroborates well with recent studies that have shown this factor to be a major contributor to the acceleration of dengue virus dispersal (and hence disease distribution in space and time), especially between urban/semi-urban and rural communities. Human migration allows multiple exposure to *Aedes aegypti* bites among migratory people; in other words, mobile persons have a greater chance of coming into close contact with various bites at multiple locations, especially in public spaces. In this study, we have concluded that the movement of local people between ecotopes is capable of increasing the risk for dengue emergence, thus further enhancing dengue transmission dynamics among the ecotopes lying between urban and semi-urban localities. Years of schooling longer than 5 years and dengue infection also remained significantly associated in the multivariate model, which suggests that higher education produces a better opportunity for people to get skilled careers, which normally are available in the commercial zones. Accordingly, these career opportunities lead to the movement of people seeking jobs far away from their hometown or community, which increases their risk of getting a dengue viral infection from the place where they have moved to work. If infected, these persons could then transmit the dengue virus to their family members and/or others around their home.

## CHAPTER VI

### CONCLUSION

Since any level of a human ecosystem is a result of interactions among people, natural components, and processes, dengue emergence marks the kinship of the human host, dengue virus pathogen, and *Aedes* mosquito vector, shaped by environment. In this inquiry, dengue transmission dynamics in urban and semi-urban ecosystems have been portrayed in points related to the eco-bio-social drivers responsible for *Aedes* mosquito vector abundance and hence the transmission of dengue.

This research first described study cluster characteristics in terms of socio-demographic, basic infrastructure, and geographical features—all of which affect dengue vector ecology among 4 diverse ecotopes and successively give rise to dengue infection among inhabitants. Marked differences in the socio-economic status (SES) of the population, as well as housing conditions, were observed between urban and semi-urban settings and among the ecotopes. The SES in the urban area was higher than that in the semi-urban area. Among all 4 ecotopes, the commercial ecotope had the highest SES, whereas the DENPURA ecotope had the lowest. The other 2 ecotopes were considered as middle economic stratum. In similar fashion, the housing structures and conditions of households in urban setting were in better condition than those seen in semi-urban setting. The conditions observed in the four ecotopes varied from the highest to lowest scales. Fair housing conditions were found in the RCDENPURA and RC ecotopes, while the commercial and DENPURA ecotopes had the highest and lowest scales, respectively.

The basic infrastructural services of all 12 study clusters have been described also with respect to electricity, water supply service, and garbage management. Every house situated in study site had fully access to electricity. Except for those in the DENPURA ecotope with a low degree of dengue transmission, all households received public water supply and garbage collections services from the

waterworks authorities, the local municipalities, and/or private companies. Despite almost all households having access to public water supply systems, piped water usage was often an unpleasant experience due to the muddiness of the water and supply interruptions, resulting from leakage of city water pipes. As a result, the local population often stored water in various containers, thereby facilitating reproduction of the *Aedes* mosquito vector. Moreover, research evidence showed that even when adequate garbage collection services were available, local inhabitants especially in DENPURA and RCDENPURA still threw rubbish around their houses and public areas.

The final cluster characteristic taken into consideration for an analysis was the geographical feature. The most relevant observations were that households in the urban locality were more contiguous than those in the peri-urban setting, and that there was intensive exploitation of lands and dense dwellings in the urban areas. Out of the 4 ecotopes, the DENPURA and commercial areas were found to exhibit high settlement of buildings together with public spaces and hence less green areas. In conclusion, even if some common cluster characteristics (for example, irregular water supply) remain among the diverse ecotopes, there is considerable heterogeneity in the SES and geographical features between the two degrees of urban settings, as well as among the four typical ecotopes. No matter how these characteristics may change, one aspect all share are the problems that directly hinder dengue prevention and control practices.

An ecological perspective can provide foresight into the appropriateness of dengue control efforts, give answers to unexpected vector control responses, and contribute to effective management solutions in an ever-changing environment. For this study, a novel ecosystem concept pertinent to ecotope has been created and applied, so as to exhaustively scrutinizing its association with *Aedes* vector abundance and the transmission of dengue fever in the study area. Besides ecotope as an ecological predictor, the degree of urbanization (urban versus semi-urban) and the intensity of dengue transmission were other determinants used to predict risk for increases in *Aedes* mosquito abundance and dengue transmission. It was found that urban and semi-urban areas as predictors posed neither a risk for an increase in vector abundance nor a dengue transmission epidemic. In contrast, settings with a high

degree of dengue transmission corroborated with an increased risk for dengue transmission but not dengue vector density. Only the ecotope of RCDENPURA manifested a strong association with dengue transmission risk. Moreover, the higher significant association shown between this ecotope and dengue transmission was demonstrated when individual ecotopes in high dengue transmission areas were applied. With respect to predictors for vector abundance, none of the 4 ecotopes contributed to an increase in vector abundance. But an analysis of the association between individual ecotopes in high dengue transmission localities and *Aedes* vector density demonstrated that the 2 ecotopes of RCDEPURA and RC become significant predictors for vector density.

Preventing and diminishing dengue virus transmission relies on the control of mosquito vectors or the interruption of human-vector contact. This study has used entomologic surveillance for operational and research purposes to estimate risk for virus transmission and disease, as well as for monitoring and evaluating vector control activities. The entomological data obtained from two different seasons (wet and dry) demonstrated clearly that the 50-400L jar and cement bath basins were the two most common water container types among all 12 study clusters in the 4 various ecotopes. In both seasons, most potential containers in all ecotopes held piped water and were used in the past 7 days; in addition, domestic households situated in the study area depended mainly on tap water.

With regard to vector ecology, most water container locations were distant from vegetation, inside the house, and in a fully shaded area. Similar to the above findings, the most used water container types in all ecotopes were 50-400L jars and cement bath basins. These containers were associated to their locations (inside the house and shaded area) and household usages (piped water, without intervention, and cover). The highest number of pupae per person (PP) in the wet season was observed in the DENPURA and RC ecotopes, while in the dry season the highest number was found in RC and RCDENPURA. Therefore, based on these lines of research evidence, we can conclude that in the wet season, the two ecotopes of DENPURA and RC pose the greatest risk for dengue virus transmission, while in the dry season, RC and RCDENPURA pose the highest risk.

Epidemiologic patterns and transmission dynamics of dengue/dengue haemorrhagic fever have proved problematic for public health personnel and scientists to understand, especially in urban and semi-urban settings. This study thus has proposed a novel approach toward a better understanding of these dynamics and patterns. The principal findings of mosquito blood analysis have been concluded in terms of dengue seroprevalence in relation diverse ecotopes and seasons, and with respect to its application. In the wet season, urbanization was not associated with past and active dengue occurrence. For degree of dengue transmission, the high dengue transmission ecotope posed a significant risk for active and past dengue infection. Moreover, the same high transmission ecotope doubled the risk for a combination of past and active dengue occurrence. In addition, the ecotope of DENPURA was a significant predictor of active dengue transmission, as well as for a combination of past and active dengue transmission. Meanwhile the RCDENPURA and RC ecotopes posed the highest risk only for a combination of past and active dengue transmission. In the dry season, urbanization was not associated with either past or active dengue occurrence, whereas the degree of dengue transmission was positively associated with active and past dengue infection, as well as a combination of the two. For this season, the RCDENPURA ecotope had the greatest risk for active dengue transmission.

Finally, DENV IgM and DENV IgG seroprevalences have been assessed with respect to seasonal pattern. The research findings have been summarized in terms of three predictors: urbanization, degree of dengue transmission, and ecotope. In the dry season, both DENV IgM and DENV IgG seroprevalence posed the most risk for dengue virus transmission, compared to the wet season. When blood meal analysis was linked with actual transmission in both seasons, the number of positive DENV IgM and DENV IgG correlated well with the number of reported dengue cases from the studied clusters during the same period of time. Although this study was limited with regards to the mosquito population samples representing dengue herd immunity of a human population in an ecotope/setting, the findings do suggest that mosquito blood meal analysis could be used as an alternative approach for seroprevalence survey to assess herd immunity for dengue fever in human populations.

Since there are no vaccines for dengue viruses, the only viable means to reduce disease incidence is control of its principal mosquito vector, *Aedes aegypti*. Vector control efforts, on the other hand, require public involvement in terms of knowledge and awareness of the vector and disease, as well as the practices adopted against *Aedes* infestation and dengue virus infection. The sustainability of dengue control and the empowerment of respective stakeholders remains a continual challenge. This Ph.D. study has thus used social science research to examine the socio-demographic parameters underlying dengue transmission and the respective stakeholders involved in dengue and vector control problems.

Samples from both high and low degree dengue transmission settings exhibited similarities in basic socio-demographic backgrounds. The preliminary investigation of dwellers with a past history of dengue infection confirmed that dengue case prevalence and epidemic activity was greatest in the high dengue transmission area, compared to the low dengue transmission area. It was found that housing structures with concrete/stone/brick in conjunction with behavior such as improper garbage management and poor practices relating water storage were possible causes for increases in vector density and likewise dengue transmission, especially in a high endemic locality. A considerable proportion of the respondents in the low dengue transmission area possessed sufficient overall knowledge about dengue, whereas respondents in the high dengue transmission area did not. With regards to sources of information on dengue, hospitals and health workers were the two most commonly cited sources for the two areas. However, neighbors seemed to be another important source for people in the high dengue transmission area, whereas radio appeared to play significant role in relaying dengue information to people in the low transmission locality. This suggests that dengue awareness activities should be emphasized in high dengue transmission areas, but the means of disseminating dengue-related knowledge ought to be in accordance with the significant information source of each area.

The findings related to vector control practices clearly showed that indoor spraying and fogging was intensively used in the high endemic area, while preventive measures were markedly applied in the low endemic area. Vector control programs need to take into account these underlying socio-demographic factors and their

prevention in order to be effective. Our stakeholder analysis in light of functions and interests provided much more insight into vector control problems and the persistence of dengue in the study area. The research findings showed that a majority of the stakeholders involved in the dengue problem and vector control were public health service offices. Applying the Social Analysis CLIP, a majority of the stakeholders were identified and profiled as upper class (dominant), while some stakeholders were characterized as forceful, influential (middle class), and marginalized (lower class). These findings clearly show that the dengue control activities in this area are mainly driven by public health authorities, who are directly in charge, whereas other respective stakeholders (including waterworks authorities, water supply companies, and public works and urban planning office) rarely participate in control programs despite their involvement. While the provincial public health office is actually the main organization responsible for dengue control, greater success will be achieved if all partners either directly or indirectly involved in the dengue problem begin to work side by side and share their resources, especially in terms of irregular water supply and garbage management.

Lastly, an integrated analysis of the eco-bio-social drivers responsible for the dengue transmission dynamics in this urban locality has led to several conclusions and recommendations. First, it was found that the ecotope of RCDENPURA clearly exhibits the highest risk for dengue incidence. This suggests that dengue control programs could successfully focus on this kind of ecotope embedded in the urban ecosystem or elsewhere, especially during an economic crisis and/or when there is less budget for such programs. Second, our research showed marked differences in sets of pivotal socio-demographic factors embedded among the diverse 4 ecotopes examined, which strongly suggests that gaining foresight into the significant socio-demographic parameters underlying increases in vector abundance and/or dengue virus transmission will significantly help the development and implementation of effective and sustainable dengue prevention and control programs. Third, it was discovered that the two strongest socio-demographic predictors of dengue transmission (especially in RCDENPURA ecotope) are movement during last 3 months and years of schooling longer than 5 years. Understanding movement as a predictor can facilitate the

identification of key individuals and areas in relation to dengue transmission dynamics, providing targets for surveillance, intervention, and improved disease prevention. Ultimately, all significant variations of eco-bio-social determinants should be taken into consideration when formulating local dengue prevention/control programs, so as to increase efficiency and maximize the potential for success.

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## **APPENDICES**

## APPENDIX A

### HOUSEHOLD QUESTIONNAIRE

**1. SURVEY INFORMATION**

T	H	H	C	C	D	D	M	M	
Team No.....	Household No.....	<b>QN CODE</b>							
Date of Survey...../...../.....									
<b>Chachoengsao Province</b> District Name..... <input type="checkbox"/> Community Name.....									
Cluster No.....	<input type="checkbox"/> Household Address.....								
Community Type	<input type="checkbox"/>	1.Densely Populated Urban Residential Area (DENPURA)				<input type="checkbox"/>	2.Commercial Area		<input type="checkbox"/>
	<input type="checkbox"/>	3.Residence				<input type="checkbox"/>	4.Mixed: Specify.....		

**2. HOUSHOLD GEOREFERENCE**

GPS Code.....	2.1 Latitude (X-UTM).....	N							
	2.2 Longitude (Y-UTM).....	E							

**3. HEAD OF HOUSEHOLD AND RESPONDENT**

**3.1 Head of Household**

3.1.1 Sex	<input type="checkbox"/>	1.Male 2.Female	<input type="checkbox"/>
3.1.2 Age (Year)	<input type="text"/>		<input type="text"/>
3.1.3 Religion	<input type="checkbox"/>	1.Muslim 2.Buddhist 3.Hindu 4. Christian 5.Other Religion 9.None	<input type="checkbox"/>
3.1.4 Years of schooling	<input type="text"/>		<input type="text"/>
3.1.5 Highest school degree attained	<input type="checkbox"/>	1.Elementary School 2.Middle School 3.High School/University 9.None	<input type="checkbox"/>
3.1.6 Occupation	<input type="checkbox"/>	1.Unemployed 2.Self-employed 3.Student 4.Housewife 5.Labourer 6.Business Person 7.Agriculturist 8.Professional 9.Others	<input type="checkbox"/>

**3.2 Respondent**

3.2.1 Sex	<input type="checkbox"/>	1.Male 2.Female	<input type="checkbox"/>
3.2.2 Age (Year)	<input type="text"/>		<input type="text"/>
3.2.3 Religion	<input type="checkbox"/>	1.Muslim 2.Buddhist 3.Hindu 4. Christian 5.Other Religion 9.None	<input type="checkbox"/>



5.6 How far is the nearest house in meters?

5.7 For how many years have you been living in this neighborhood?

5.8 For how many years have you been living in this house?

5.9 How many doors does the house have?

5.10 How many windows does the house have?

5.11 How many windows with screen?

5.12 How many windows with curtain?

5.13 How many windows closed permanently?

5.14 Do you have a back yard or open space attached to your house?  1.Yes 2.No

5.15 If yes, how many trees or bushes taller than adult person are in your yard?

5.16 What is your main water supply?  1.Piped supply 2.Well water 3.Hand pump 4.spring 5.Bore well 6.River water 7.Water tanker 9.Others...

5.17 Do you store water?  1.Yes 2.No

5.18 For which purposes do you store water? (Interviewer: ask one by one and enter answer)

1. For washing

2. For cleaning the house

3. For drinking

4. For all of these

5. For others.....

5.19 If you store water, how frequently do you empty the following containers? 1.Every day 2.Alternate day 3.Every week 4.Every month 5.Not usually every month

1. Large size containers (over 200 liters)

2. Medium size containers (cement tank, etc)

3. Small size containers (buckets, jars, etc)

5.20 What kind of toilet do you mainly use?  1.Toilet inside house 2.Toilet outside house 3.Septic tank 4.Community latrine 5.Latrine shared by others 6.No latrine; Open air

5.21 How is the solid waste and rubbish disposed?  1. Collected by a man/a truck   
 2. Emptyng in a particular site  
 3. No fixed pattern  
 4. Others.....

**6. KNOWLEDGE ABOUT DENGUE AND ITS VECTOR**

6.1 Have you ever heard about dengue?  1. Yes 2. No

6.2 If yes, from where have you heard about dengue? *1. Television 2. Radio*  
 (Ask for the two most important sources) *3. Newspaper 4. Health worker*

6.2.1 The first important source  *5. Friend/Peer 6. Neighborhood*   
*7. Poster 8. From hospital*

6.2.2 The second important source  *9. Others.....*

6.3 Have you ever received suggestions or participated in any training for dengue prevention and control activities  1. Yes 2. No

6.4 How does dengue spread from one person to another?  
 (Do not read the answers but let people say spontaneously)

6.4.1 Mosquito  1. Mentioned 2. Not Mentioned

6.4.2 Direct contact  1. Mentioned 2. Not Mentioned

6.4.3 Water  1. Mentioned 2. Not Mentioned

6.4.4 Flies  1. Mentioned 2. Not Mentioned

6.4.5 Dirty environment  1. Mentioned 2. Not Mentioned

6.4.6 Rats, birds, pigs, other animals  1. Mentioned 2. Not Mentioned

6.4.7 Others.....  1. Mentioned 2. Not Mentioned

6.5 Can dengue be prevented?  1. Yes 2. No   
 3. Uncertain/Don't Know

6.6 How do mosquitoes develop?  1. From eggs 2. From larvae   
 3. From both eggs and larvae  
 4. Others.....

6.7 Where do the mosquitoes generally lay their eggs or breed?

6.7.1 Clean water  1. Mentioned 2. Not Mentioned

6.7.2 Dirty water  1. Mentioned 2. Not Mentioned

6.7.3 Any water  1. Mentioned 2. Not Mentioned

6.7.4 On wet clothes  1. Mentioned 2. Not Mentioned

6.7.5 Plants  1. Mentioned 2. Not Mentioned

6.7.6 Don't know  1. Mentioned 2. Not Mentioned

6.7.7 Others.....  1. Mentioned 2. Not Mentioned

6.8 Have you ever seen the larvae in your water?  1. Yes 2.No   
3.Uncertain 4.Don't know

6.9 Does it matter if one has mosquito larvae  1. Yes 2.No   
in the household water containers? 3.Uncertain 4.Don't know

## 7. ATTITUDE RELEVANT TO DENGUE AND VECTOR CONTROL

### 7.1 ATTITUDE ABOUT DENGUE

Is dengue in your opinion a serious or  1.Serious 2.Mild   
a mild disease? 3.May be both 9.Don't know

### 7.2 ATTITUDE ABOUT ROLES OF FAMILY, GOVERNMENT, AND GENDER *Interviewer informs the purpose and attitude scales before starts interviewing*

*Scale: 5.Strongly Disagreed 4.Agreed 3.Unsure 2.Disagreed 1.Strongly Disagreed*

7.2.1  It is important to clean the house environment regularly.

7.2.2  It is necessary to clean water containers once a week.

7.2.3  It is difficult to remove mosquito breeding places.

7.2.4  It is not necessary for neighbors to cooperate to prevent dengue.

7.2.5  Only families with dengue should participate in dengue prevention.

7.2.6  The government is doing enough to prevent dengue.

7.2.7  The health workers have a significant role in dengue prevention  
at community level.

7.2.8  Women are not mainly responsible for cleaning water containers in household.

7.2.9  Men clean large containers in households.

7.2.10  Women are responsible for cleaning household environment.

7.2.11  Men should make a decision for spending money to control mosquitoes  
in household.

7.2.12  Households with mosquito larvae should be fined.

7.2.13  Health workers are solely responsible for removing mosquito breeding sites.

7.2.14  It is not right to pour away stored water once a week to clean  
the large and medium size containers.

**8. VECTOR CONTROL PRACTICES**

8.1 What do you do at home to reduce the nuisance of mosquitoes?

*(Ask one by one and tick the answer)*

- 8.1.1 Nothing  *1. Yes 2.No*
- 8.1.2 Indoor spraying  *1. Yes 2.No*
- 8.1.3 Cleaning rubbish  *1. Yes 2.No*
- 8.1.4 Covering the water containers  *1. Yes 2.No*
- 8.1.5 Putting chemicals in water  *1. Yes 2.No*
- 8.1.6 Putting fish in water  *1. Yes 2.No*
- 8.1.7 Killing mosquitoes; e.g. with chemicals  *1. Yes 2.No*
- 8.1.8 Personal protection with repellents  *1. Yes 2.No*

8.2 What does the government do against mosquito?

*(Do not read the answers but let people say spontaneously)*

- 8.2.1 Checking the water containers  *1.Mentioned 2.Not Mentioned*
- 8.2.2 Adding chemicals in water  *1.Mentioned 2.Not Mentioned*
- 8.2.3 Spraying inside houses  *1.Mentioned 2.Not Mentioned*
- 8.2.4 Educating people  *1.Mentioned 2.Not Mentioned*
- 8.2.5 Supplying lid covers  *1.Mentioned 2.Not Mentioned*
- 8.2.6 Giving fish to put in water  *1.Mentioned 2.Not Mentioned*
- 8.2.7 Fogging  *1.Mentioned 2.Not Mentioned*
- 8.2.8 Cutting the plants  *1.Mentioned 2.Not Mentioned*
- 8.2.9 Others.....  *1.Mentioned 2.Not Mentioned*
- 8.2.10 Nothing  *1.Mentioned 2.Not Mentioned*

8.3 When did you have last time the visit by a health inspector?

- 1.During last month*
- 2.Two to six months ago*
- 3.Six to twelve months ago*
- 4.More than a year ago*
- 5.Do not remember/ Never*

8.4 Have you ever received support or materials to prevent dengue?

- 1. Yes 2.No*

8.5 If yes

- Specify.....*

8.6 Is there any community effort to clean the environment?

- 1. Yes 2.No 3.Don't know*

8.7 If yes. What does the community do to get rid of dengue mosquitoes?  1. Eliminate breeding places   
 2. Visit households to ensure programme implementation  
 3. Others.....

8.8 What can you do to prevent mosquito breeding in and around your house?  
 (Ask one by one and tick the answer)

8.8.1 Covering all the water containers  1. Yes 2.No

8.8.2 Changing water once a week  1. Yes 2.No

8.8.3 Eliminating stagnant water  1. Yes 2.No

8.8.4 Brushing and cleaning the inner surface of water containers  1. Yes 2.No

8.8.5 Putting larva eating fish  1. Yes 2.No

8.8.6 Putting chemicals into the container  1. Yes 2.No

8.8.7 Removing larvae  1. Yes 2.No

8.8.8 Removing rubbish  1. Yes 2.No

8.8.9 Others.....  1. Yes 2.No

8.9 What should the government do for improving dengue control?  
 (Do not read the answers but let people say spontaneously)

8.9.1 Checking the water containers  1. Mentioned 2. Not Mentioned

8.9.2 Adding chemicals in water  1. Mentioned 2. Not Mentioned

8.9.3 Spraying inside houses  1. Mentioned 2. Not Mentioned

8.9.4 Educating people  1. Mentioned 2. Not Mentioned

8.9.5 Supplying lid covers  1. Mentioned 2. Not Mentioned

8.9.6 Giving fish to put in water  1. Mentioned 2. Not Mentioned

8.9.7 Fogging  1. Mentioned 2. Not Mentioned

8.9.8 Cutting the plants  1. Mentioned 2. Not Mentioned

8.9.9 Others.....  1. Mentioned 2. Not Mentioned

8.9.10 Nothing  1. Mentioned 2. Not Mentioned

**MANY THANKS FOR YOUR PATIENCE**

(.....)  
 Signature of interviewer

(.....)  
 Signature of supervisor

## **APPENDIX B**

### **STAKEHOLDER ANALYSIS MATRIX FORM**

**Stakeholder analysis matrix for the “eco-bio-social” research initiative:  
functions and interests of stakeholders**

**Study Site:**

**Principal Investigator:**

**Researcher filling out this matrix:**

**Date of finalization of the matrix:**

**This stakeholder matrix aims to collect information about stakeholders in all study sites with the key questions**

- 1) who does what
- 2) funding: how much, where from
- 3) collaborating with whom
- 4) influence within stakeholder group and on activities
- 5) Perceived potential for additional involvement in dengue activities

This information will be used to link the activities in the research projects with the analysis of this stakeholder matrix.

**Please use two rows for each stakeholder:**

- 1) Information received from the stakeholder of the respective agency/group (Information from the stakeholder)
- 2) Further comments/impressions of the researcher filling out of the matrix (Researchers impression)

Stakeholder	Main mission or activities of stakeholder	Main activities in relation to dengue	Budget in US \$ last year	Source of funding	Which proportion of your work is on dengue?	Established collaboration with other stakeholders	Please mention any particular success or problem	Any other item
1) Information from the stakeholder: please list each stakeholder group in one row with the full original name (see note* below) 2) Researchers impression (see note **below)	(this may not be dengue)	(see note *** below)	(if available otherwise mark "unknown")	(e.g. MoH, if available, otherwise mark "unknown")	(see note **** below)	(please list the other stakeholders)		
<i>Example</i> 1) Information from the stakeholder "Healthy Colombo initiative"	Our main mission is on dengue control COMBI is main strategy component	Health Promotional Program	400 000 US \$ in 2009	MoH	High	MoH Dengue vector control programme	Our biggest success is that we are embedded in the community	We will cease to exist after this year because of lack of funding
2) Researchers impression								
1) Information from the stakeholder: Full name of the agency/group::								
2) Researchers impression								
1) Information from the stakeholder: Full name of the agency/group::								
2) Researchers impression								
1) Information from the stakeholder: Full name of the agency/group::								
2) Researchers impression								
1) Information from the stakeholder: Full name of the agency/group::								
2) Researchers impression								

Note\*:  
 Stakeholders:  
 This could be Ministry of Public Health, Municipality, State Government, Urban Development Agency, Tire industry, NGO A, Community group A, Community group B, Agency A, Agency B, University, WHO, local governance structure A, religious groups Potential stakeholders, or stakeholders who are not collaborating, but working parallel should also be included (e.g. municipal water supply office: this agency may have never thought about dengue; nor waste collectors, etc.....)

Note\*\*:  
 Researchers impression  
 Please describe in your own words

- if in your opinion this stakeholder is more collaborating, or more working in parallel with other stakeholders
- if in your opinion the stakeholder has influence/power in the entire group of stakeholders
- any other item you would want to mention

Note\*\*\*:  
 Main activities in dengue, this may be:

- Entomological activities: vector control, vector surveillance
- Health service activities: health service management, disease surveillance, health promotion
- Other services with related activities: urban development, water management, waste management
- Collaborative activities, social mobilization, communication
- Support activities, finances, human resources, capacity training, innovation/knowledge management/research, regulatory (legislation), monitoring and evaluation
- Any other activities

Note\*\*\*\*:  
 Which proportion of your work on dengue?

- High: main focus of activities on dengue and highly involved
- Medium: one of many different health activities, but highly involved in dengue control
- Low: one of many different health activities and not much involved in dengue control

## APPENDIX C

### SURVEY FORM OF CLUSTER BACKGROUND INFORMATION

**1. GENERAL INFORMATION**

Team No..... **SF CODE**

T	C	C	D	D	M	M

Date of Survey...../...../.....

**Chachoengsao Province** District Name.....

Community Name..... Cluster No.....

Geographical coordinates of cluster

GPS Code..... Latitude (X-UTM).....N 

--	--	--	--	--	--	--	--

Longitude (Y-UTM).....E 

--	--	--	--	--	--	--	--

Length.....meter (North-South) Breadth.....meter (East-West)

Total area.....square meters

**2. DENSITY OF POPULATION AND GREEN AREAS**

2.1 Socio-economic characteristics of population living in cluster  *1.Upper economic stratum*   
*2.Middle economic stratum*  
*3.Lower economic stratum*  
*4.Mixed:Specify.....*

*Comments.....*

2.2 Housing conditions  *1.Very good 2.Good*   
*3.Satisfactory 4.Poor*  
*5.Mixed:Specify.....*

*Comments.....*

2.3 Residential function  *1.Residential only*   
*2.Residential and commercial*  
*3.Residential with small, private manufacturing industry*  
*4.Mainly commercial with few residence*

*Specify proportion.....*

*Comments.....*

2.4 Type of houses 1.Mainly single-floor   
2.Mainly single-floor elevated  
3.Mainly double-floor  
4.Mainly single-floor rowed  
5.Mainly double-floor rowed  
6.Others.....

*Specify proportion*.....

*Comments*.....

2.5 Approximate mean distance between houses.....meter     .     
*(from GPS measurement)*  
*What is the sampling procedure?*.....

*Comments*.....

2.6 Do the majority of the houses (>75 %) have  1. Yes 2.No   
 typical gardens/patios or land or courtyard?

*Comments*.....

2.7 Is the cluster largely shaded?  1. Yes 2.No

2.8 If yes: specify degree of shading  1.High 2.Moderate 3.Low

*Comments*.....

2.9 Are there open spaces?  1. Yes 2.No

2.10 If yes: specify number of space(s).....

2.11 If yes: what is the percentage of  1. <25% 2. 25-50%   
 vegetation covered? 3. 50-75% 4. >75%

2.12 If yes: what is the percentage of  1. <25% 2. 25-50%   
 trees, shrubs, and grasses covered? 3. 50-75% 4. >75%

*Comments*.....

2.13 How many large puddles      
*(larger than 10 sqm) are there in the cluster?*

*Comments*.....

2.14 Are there garbage dumps?  1. Yes 2.No

2.15 If yes: specify number and size

*Comments*.....

**3. BASIC INFRASTRUCTURE OF THE CLUSTER**

3.1 Is there piped water supply?  1. Yes 2.No

3.2 If yes: specify type and number

3.1 Common source with no pipe

3.2 Deep well with piping for distribution

3.3 Public treated water supply

Comments.....

3.3 Availability of electricity  1. Yes 2.No

3.4 If yes: Specify percentage of house and building with electric supply.....%

Comments.....

3.5 Type of impervious surface area  1.Mainly concrete   
2.Soil 3.Others.....

3.6 Specify percentage of the area  1. <25% 2. 25-50%   
3. 50-75% 4. >75%

Comments.....

3.7 Are there any tire capping facilities?  1.Yes 2.No

3.8 If yes: specify number

Comments.....

3.9 Frequency of solid waste disposal  1.Less than one per week   
2.Once per week  
3.More than once per week

3.10 Schedule of pick up day and time  
Day..... Time.....

Comments.....

**4. PUBLIC SPACES (INCLUDE BOTH GOVERNMENT AND PUBLIC PRIVATE)**

4.1 Number of schools in the cluster

4.1.1 Public school

4.1.2 Private school

Comments.....

4.2 Are there any religious sites?  1.Yes 2.No

4.3 If yes: specify type and number

Comments.....

4.4 Is there any market?  1.Yes 2.No

4.5 If yes: specify type, size, and number  
*Size : 1.Large 2.Medium 3.Small*

4.5.1 Public market; size.....

4.5.2 Private market; size.....

*Comments*.....

4.6 Is there any railway/ bus station?  1.Yes 2.No

*Comments*.....

4.7 Is there any public/private cemetery?  1.Yes 2.No

*Comments*.....

4.8 Is there any ferry/jetty?  1.Yes 2.No

*Comments*.....

4.9 Is there any public/private health facility?

4.10 Is yes: specify number

*Comments*.....

4.11 Is there any cinema/theater hall?  1.Yes 2.No

4.12 Is there any commercial mall?  1.Yes 2.No

4.13 Is there any other public place?  1.Yes 2.No

4.14 If yes: specify type and number

*Comments*.....

**5. RECENT VECTOR CONTROL ACTIVITIES**  
**(According to vector control office)**

5.1 Was there any interventions for vector control during the preceding 3 months?  1.Yes 2.No

*Comments*.....

5.2 If yes: which intervention(s)?

5.2.1 Routine larval survey  1.Yes 2.No

5.2.2 Fogging  1.Yes 2.No

5.2.3 Focal intervention triggered by case  1.Yes 2.No

5.2.4 Others.....  1.Yes 2.No

**6. COMMUNITY PARTICIPATION**

6.1 Is there any kind of community work?  1.Yes 2.No

6.2 If yes: specify type of community meeting  
*Comments*.....

6.3 Were there any community meetings  1.Yes 2.No   
during the last 3 months?

**7. ADJACENT AREAS OF THE CLUSTER  
(Any side/perimeter of the cluster)**

7.1 What is the land cover/ land use surrounding area?  
.....  
.....

7.2 Presence of lake/ puddles  1.Yes 2.No

7.3 If yes: specify locality.....  
*Comments*.....

7.4 Presence of vegetated land cover  1.Yes 2.No

7.5 If yes: specify locality.....  
*Comments*.....

7.6 Any presence of tire capping facility?  1.Yes 2.No

7.7 If yes: specify locality.....  
*Comments*.....

7.8 Any garbage dumping areas?  1.Yes 2.No

7.9 If yes: specify locality.....  
*Comments*.....

**8. NARRATIVE DESCRIPTION OF CLUSTER:**

8.1 Aspect 1  
.....  
.....

8.2 Aspect 2  
.....  
.....

8.3 Aspect 3  
.....  
.....

**9. IMPRESSION OF PUBLIC SPACE  
(According to dengue transmission)**

.....  
.....  
.....  
.....  
.....  
.....  
.....  
.....  
.....  
.....

(.....)  
*Signature of the team leader*

## APPENDIX D

### ENTOMOLOGICAL SURVEY FORM FOR HOUSEHOLD

Team No..... Household No..... **ESFH CODE**

T	H	H	C	C	D	D	M	M

  
 Date of Survey...../...../..... Inspector.....  
**Chachoengsao Province** District Name..... Community Name.....  
 Cluster No..... Household Address..... Name of owner.....  
 Number of family member..... Climatologic records *Indoor* T.....°C RH .....%  
*Peripheral* T.....°C RH .....% *Outdoor* T.....°C RH .....% Remark.....

Container number	Container code [A]	Larvae/Pupae (0=Not found, 1=Found)	Water capacity volume (L)	Water volume [B]	Type of water (1=tap/well, 2=rain, 3=other)	Purpose of storing (1=drink, 2=use, 3=1+2, 4=not)	During-past-7-day usage (1=Yes, 2=No)	Under vegetation (1=fully, 2=partially, 3=not)	Container location (1=Inside, 2=Outside)	Shade (1=fully, 2=partially, 3=not)	Intervention [C]	Cover (1=preventable, 2=partially, 3=not)	Larval Count				Pupal Count		
													Absolute number	Sample	Estimated	Larval density (0=no larva, 1=1-10, 2=11-50, 3>50)	Absolute number	Sample	Estimated
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
12																			
13																			
14																			
15																			
16																			
17																			
18																			
19																			
20																			

[A] **Container code:** A1.1= $\geq$ 1,000L Cement tank A1.2=50-100L Cement tank A2=Circle cement tank  
 A3.1=200L Earthen jar A3.2=50-150L Earthen jar A3.3=400L Large jar A3.4=1,000L Large jar  
 A4=Small local jar A5=200L Drum A6=Bucket A7=Flower vase  
 A8.1=Bowl for ant trap A8.2=Pet dish A8.3=Flower vase saucer A8.4=Lotus bowl  
 A9.1=Bottle/Can A9.2=Plastic container A9.3=Broken jar A10=Used tire  
 A11=Coconut shell A12=Natural container A13=Other..... A14=Other.....

[B] **Water volume:** 1=Fully 2=3/4 3=1/2 4=1/4 5=Scantly

[C] **Intervention:** 1.1=Temephos 1% 1.2=BTI 1.3=Brushing & Cleaning 1.4=Covering 2=None

**APPENDIX E**  
**ETHICAL REVIEW AND APPROVAL (THESIS FOR Ph.D.)**



COE. No. MU-IRB 2010/019.2505

**Certificate of Exemption from Ethical Review**  
**Mahidol University Institutional Review Board**

**Title of Project.** An Eco-Bio-Social Approach to Assess Dengue Transmission Dynamics in Thailand  
(Thesis for Ph.D)

**Principal Investigator.** Mr. Surachart Koyadun

**Name of Institution.** Faculty of Science

Mahidol University Institutional Review Board is in full compliance with International Guidelines for Human Research Protection such as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

**Date of Determination.** 25 May 2010

**Signature of Chairman.** .....  .....  
(Professor Shusee Visalyaputra)

**Signature of Head of the Institute.** .....  .....  
(Associate Professor Sansahee Chaiyaroj)  
Vice President for Research and Academic Affairs

**APPENDIX F**  
**ETHICAL REVIEW AND APPROVAL (RESEARCH PROJECT)**



COA. No. MU-IRB 2009/162.2307

**Documentary Proof of Mahidol University Institutional Review Board**

**Title of Project.** Socio-Bio-Ecosystem Approach to Dengue Prevention and Control in Thailand

**Principle Investigator.** Associate Professor Dr. Pattamaporn Kittayapong

**Name of Institution.** Faculty of Science


**Approval includes.**

- 1) Annual Report version received date 17 July 2009
- 2) Participant Information Sheet for Entomology & Survey version date 17 July 2009
- 3) Participant Information Sheet for KAP Survey version date 17 July 2009
- 4) Informed Consent form for Entomology & Survey version date 17 July 2009
- 5) Informed Consent form for KAP Survey version date 17 July 2009
- 6) Questionnaire version received date 17 July 2009

Mahidol University Institutional Review Board is in full compliance with International Guidelines for Human Research Protection such as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

**Date of Renwal (1<sup>st</sup>).** 23 July 2009

**Date of Expiration.** 22 July 2010

**Signature of Chairman.** .....   
 (Professor Shusee Visalyaputra)

**Signature of Head of the Institute.** .....   
 (Associate Professor Sansanee Chaiyaroj)  
 Vice President for Research and Academic Affairs

Office of the President, Mahidol University, 999 Phuttamonthon 4 Rd., Salaya, Phuttamonthon District, Nakhon Pathom 73170. Tel. (662) 8496223-5 Fax. (662) 8496223

## BIOGRAPHY

<b>NAME</b>	Mr. Surachart Koyadun
<b>DATE OF BIRTH</b>	22 October 1968
<b>PLACE OF BIRTH</b>	Phangnga Province, Thailand
<b>INSTITUTIONS ATTENDED</b>	Sukhothai Thammathirat University, 1990-1992 Bachelor of Public Health Sukhothai Thammathirat University, 1993-1996 Bachelor of Public Health (Public Health Administration) Mahidol University, 1997-1999 Master of Science (Public Health) Major in Infectious Diseases Mahidol University, 2006-2010 Doctor of Philosophy (Biology)
<b>JOB TITLE</b>	Professional-Level Public Health Technical Officer
<b>EMPLOYMENT ADDRESS</b>	Office of Disease Prevention and Control 11 Nakhon Sri Thammarat Province 184/117 Tewaburi Road, Posadech Subdistrict, Muang District, Nakhon Sri Thammarat Province, 80000, Thailand
<b>PRESENTATION</b>	Koyadun, S. and Kittayapong, P. The Application of Mosquito Blood Meal Analysis to Assess Dengue Herd Immunity in Urban and Semi-urban Settings in Thailand. Oral Presentation at the 17 <sup>th</sup> European Society for Vector Ecology Conference. 13 <sup>th</sup> – 17 <sup>th</sup> September 2010 Wroclaw, Poland