The Control of Neglected Tropical Diseases: A Focus on Visceral Leishmaniasis and Dengue in Bangladesh

顧みられない熱帯病の制御：

バングラデシュにおける内臓リーシュマニア症とデング熱

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筑波大学大学院博士課程人間総合科学研究科

Farhana Ferdousi

ABSTRACT

Purpose:

The overall purpose of this study was to demonstrate effective and sustainable control strategies for visceral leishmaniasis (VL) and dengue, two of the most prevalent neglected tropical diseases (NTDs), in Bangladesh.

The role of active disease surveillance and neem extract intervention to control VL was evaluated. For dengue, containers serving as the primary breeding sites of *Aedes* larvae were identified in order to focus on targeted vector control strategies.

Material and method:

Active disease surveillance using a simple diagnostic tool, rapid rK39 dipstick test, was conducted in a VL endemic area of Bangladesh from 2006 to 2008. During the study period, neem oil solution was sprayed in the households of intervention area selected by cluster randomization, while no intervention was carried out for the control area. Socioeconomic and environmental information was also collected.

A secondary analysis of a household entomological survey of dengue was done to identify the most productive containers for *Aedes* larvae. The survey was conducted in Dhaka from August through October 2000, the peak epidemic period of dengue in Bangladesh. Field research assistants looked for the containers with standing water, and for *Aedes* larvae within the containers.

Results:

After 1 year of active disease surveillance in 2007, the number of reported VL cases was substantially increased over that of the previous year (RR = 1.38; 95% CI = 1.07 - 1.79). However, the incidence of VL in 2008 was significantly lower than in 2006 (RR = 0.19; 95% CI = 0.12 – 0.32). Neem oil was not found to be effective in the control of VL. However, the proportion of increased case reporting in 2007 was 5 times higher in control area than in intervention area. The proportion of decreased case reporting in 2008 was 2 times higher in intervention area than in control area. Factors associated with VL included younger age group (3-14 years), not having electricity in the household, never using mosquito-control measures, and never using a bed net.

A total of 9222 households and 38 777 containers were inspected for *Aedes* larvae. Overall tires, and the water reserving containers, e.g., tanks, drums, and earthen jars, were found to be the most important containers for *Aedes* larval breeding. Less importance was indicated for buckets, and discarded appliances. *Ae. aegypti* showed higher affinity for indoor containers, while *Ae. albopictus* were dominant outdoor breeder. Independent type of household, having any kind of water storage system (i.e., tanks, drums, earthen jars, and buckets) in the household, and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae.

Discussios:

The increased incidence rate of clinical leishmaniasis in 2007 may indicate that community-based active surveillance using a simple diagnostic tool would be able to substantially increase the case reporting. Early case reporting and referral for treatment could

significantly reduce the source of infection within the community, which resulted in a notably decreased incidence rate of VL in 2008. Although neem oil was not directly found to be effective in the control of VL, we may assume that neem intervention, along with active disease surveillance, played an important role to decrease the incidence rate of VL. This study also found that use of some kind of mosquito-control measures and bed nets were protective against VL. However, very few households reported using such measures regularly.

Water storage containers which usually contain large volume of water and are never emptied were consistently more likely to have *Aedes* larvae, such as earthen jars, tanks, and drums. On the other hand, containers which are relatively smaller in size and are frequently used have less chance to be infested with *Aedes* larvae. Another important breeding site is the tires. Usually tires are left abandoned. The collected rain water in tires is an ideal source of *Aedes* larvae.

Conclusion:

Although the unique disease limiting factors of VL make it a potential candidate for elimination, massive efforts in community-based active disease surveillance coupled with scaled-up personal protection measures and integrated vector management interventions are required to achieve the goal. Neem oil would be a favorable option as an environment-friendly measure to control VL among the marginalized poor of the endemic areas. However, further research evidence is required with the support of local government and international organization.

Until a vaccine, clinical cure, or genetic strategy is available, control of dengue will continue to depend on suppression of the vector populations. Generalized community clean-up campaigns of vector breeding sites have had only a transient and limited effect on disease

incidence. The identification and subsequent elimination of the most *Aedes* mosquito producing containers in a given area may potentially reduce mosquito density below a critical threshold, which could result in more efficient and cost-effective control campaigns.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

APVMA	**Australian Pesticides and Veterinary Medicines Authority**
BI	**Breteau Index**
CFR	**Case Fatality Rate**
CI	**Confidence Interval**
CI	**Container Index**
CMH	**Cochran-Mantel-Haenszel**
DALYs	**Disability–Adjusted Life Years**
DCC	**Dhaka City Corporation**
DDT	**Dichloro Diphenyl Trichloro ethane**
DEN-1	**Dengue-1**
DF	**Dengue Fever**
DGHS	**Directorate General of Health Services**
DHF	**Dengue Hemorrhagic Fever**
DSS	**Dengue Shock Syndrome**
FDA	**Food and Drug Administration**

HI	**House Index**
IBM	**The International Business Machines Corporation**
icddr,b	**The International Centre for Diarrhoeal Disease Research, Bangladesh**
IDM	**Innovative and intensified Disease Management**
ITN	**Insecticide Treated bed net**
KA	**Kala-Azar**
MEP	**Malaria Eradication Program**
MPDC	**Malaria & Parasitic Disease Control Unit**
NTDs	**Neglected Tropical Diseases**
PCT	**Preventive Chemotherapy and Transmission control**
PKDL	**Post Kala-azar Dermal Leishmaniasis**
PPM	**Parts Per Million**
RK39	**Recombinant K39**
RR	**Relative Risk**
SD	**Standard Deviation**
SPSS	**Statistical Package for the Social Sciences**
USAID	**The United States Agency for International Development**

VEM	**Vector Ecology and Management**
VL	**Visceral Leishmaniasis**
WHO	**World Health Organization**

Chapter 1

INTRODUCTION

1.1 WORLD SCENARIO

Neglected tropical diseases (NTDs) are a group of disabling infections affecting more than 1 billion people worldwide, mostly those living in remote rural areas, urban slums or conflict zones; mainly in tropical and subtropical countries. They are called neglected because they have been largely wiped out in the more developed parts of the world and persist only in the poorest, most marginalized communities. Therefore, NTDs have low profile and status in public health priorities as those who are affected are poor and have little political voice. NTDs seldom kill directly but cause lifelong misery that stunts children's growth, leaves adults unable to function to their fullest and heightens the risks of other diseases. NTDs, in particular, are "diseases of poverty". These diseases place those most at risk in an endless cycle of poverty that continues from generation to generation.

World Health Organization (WHO) has prioritized 17 most important NTDs result from 4 different causative agents: virus, protozoa, helminthes, and bacteria. These are dengue/severe dengue, rabies, chagas disease, human african trypanosomiasis (sleeping sickness), leishmaniases, cysticercosis/taeniasis, dracunculiasis (guinea-worm disease), echinococcosis, foodborne trematodiases, lymphatic filariasis, onchocerciasis (river blindness), schistosomiasis, soil-transmitted helminthiases, buruli ulcer, leprosy (Hansen disease), trachoma, and yaws. One-sixth of the world's populations suffer from one or more NTDs. Worldwide, 149 countries and territories are affected by at least 1 NTDs. These diseases tend to occur together in the same geographic cluster. The most shocking part is that 100% of low-income countries are affected by at least 5 NTDs

simultaneously. Individuals are often afflicted with more than one parasite or infection. NTDs kill an estimated 534,000 people worldwide every year.

However, NTDs can easily be prevented and managed. Treatment cost for most NTD mass drug administration programs is estimated at less than US 50 cents per person per year. Following the end of World War II, Japan led an aggressive domestic campaign against soil-transmitted helminths, lymphatic filariasis, malaria and schistosomiasis, eliminating them as public health threats within only 10 years. Therefore, controlling NTDs is a goal that we can achieve within a matter of years, not a generation or lifetime. WHO has developed technical guidelines for national programs. The guidelines emphasize a coordinated, cost-effective approach to the implementation of national elimination and control activities for the NTDs. WHO recommends 3 main strategic programs to control NTDs including preventive chemotherapy and transmission control (PCT), innovative and intensified disease management (IDM), vector ecology and management (VEM) along with improved sanitation, and health promotion. However, eliminating these diseases as public health threats by the end of the decade will require commitments of stakeholders from across government, industry and civil society as well as sustained investments (1, 2).

1.2 NTDs in BANGLADESH

Bangladesh is a vibrant developing country situated in south-Asia; the region known for large population and large number of people affected by communicable diseases. It gained independence and became sovereign in the year 1971. During the last

four decades, the country has been striving to ameliorate the socio-economic conditions and living standards of the people, and to improve the health care delivery and health status of the people.

Bangladesh is bordered by India on western, northern and eastern side. The Bay of Bengal surrounds the southern border and a small strip of land adjoins Myanmar. The total land area is 147 570 sq km (56 977 sq miles). The climate is tropical with a hot and rainy summer and a dry winter and the temperature ranges from about 26° c in January to 35° c in April. Bangladesh is a low lying country with a wide net work of rivers and rivulets and plenty of rainfall. This makes the country prone to frequent and severe floods, affecting the crops, livelihood and health of the people.

There is a huge burden of the NTDs in Bangladesh. The major NTDs prevalent in Bangladesh are lymphatic filariasis, visceral leishmaniasis (VL), dengue/severe dengue, and soil transmitted helminthiasis (3, 4). As in the other parts of the world, in Bangladesh too the NTDs impair the people's capacity very adversely and accentuate poverty. Each disease has pockets of very high prevalence and morbidity.

Among the NTDs, VL and dengue in Bangladesh were focused in the present study. Both VL and dengue are vector borne diseases. VL is transmitted by the bite of *Phlebotomus argentipes*, while dengue is transmitted through *Aedes aegypti*.

VL is highly endemic in Bangladesh. Between 1994 and 2013, a total of 109 266 VL cases with 329 deaths were reported from 37 endemic districts in Bangladesh. The Mymensingh district was the most affected with 53 582 (49.04%) cases (5). In 2005, Bangladesh, India and Nepal signed a Memorandum of Understanding for joint efforts to

eliminate this deadly disease. The target of the VL elimination program is to reduce the annual incidence of VL to less than one per 10 000 people at the district or sub district level (upazila in Bangladesh, sub district in India and district in Nepal) by 2015. However, a recent assessment of existing epidemiological surveillance data in 2013 revealed that 16 districts in Bangladesh still have the high incidence rate of VL ranging from 1.06 to 18.25 per 10 000 people per year (5). Moreover, this assessment was based on official notification data only which probably suffered from under-reporting. Therefore, urgent initiatives, including community-based active disease surveillance for early case detection and management, and effective vector control efforts, needed to be established to achieve the goal of VL elimination program.

In Bangladesh, first large scale outbreak of dengue occurred in 2000 affecting the major cities. There were 5 551 cases and 93 deaths reported. The case fatality rate (CFR) was 1.7%. Since 2000, it has become a regular phenomenon of occurrence of dengue every year. Although the case fatality rate (CFR) remains low due to the improved clinical management in the hospitals, the number of cases is sometimes high. In 2002, there were 6104 cases and 58 deaths while in 2005, there were 1048 reported cases and 4 deaths. In 2006 the number of cases and deaths increased by 2 fold as compared to 2005. In 2010, 5500 people were infected, with 98 deaths. Therefore, effective and sustainable control measures are in high priority to prevent dengue incidence (6).

In chapter 2, the prospect of active surveillance as an important element of the VL elimination program has been demonstrated. The potential role of neem extract as an environment-friendly and cost-effective vector control measure in an endemic area of

Bangladesh was evaluated. Some risk factors associated with VL incidence in this endemic community have also been identified.

In chapter 3, *Aedes* larval breeding habitats with special attention to key containers have been demonstrated in order to propose effective vector control messages specific for each key containers. Some risk factors for the households infested with *Aedes* larvae have also been identified.

1.3 CONTRIBUTION TO THIS WORK

STUDY 1: ROLE OF ACTIVE DISEASE SURVEILLANCE AND NEEM OIL INTERVENTION TO CONTROL VISCERAL LEISHMANIASIS IN BANGLADESH

This study was a collaborative research work between International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and the University of Tsukuba. The study was conducted in Bangladesh from December 2006 to December 2008. I was part of the research team for data management as a member of the Department of Clinical Trial and Clinical Epidemiology at the University of Tsukuba. For my doctoral thesis, I performed the statistical analysis of the data (Section 2.3.6) and the results obtained are presented in this thesis (Section 2.4, Table 2.1 – 2.6). Results were also published in the journal of Tropical Medicine and Health (7).

STUDY 2: IDENTIFICATION AND MANAGEMENT OF KEY CONTAINER FOR *AEDES* LARVAL BREEDING TO CONTROL DENGUE IN BANGLADESH

This study was conducted by International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) during the large outbreak of dengue in Bangladesh in 2000. The primary purpose of the study was to identify the areas with high density of *Aedes* mosquitoes in order to prevent the further transmission of dengue. As a part of my doctoral thesis, I performed the secondary analysis of the dataset collected from the above study with the aim to identify the containers that served as primary producers of *Aedes* larvae during the dengue outbreak, in Section 3.3.4, and 3.4, Figure 3.2 (a, b, c) – 3.4, and Table 3.1 – 3.4 of this thesis.

1.3 REFERENCES

1. http://www.who.int/neglected_diseases/en/. World Health Organization. Neglected tropical diseases.

2. http://www.cdc.gov/globalhealth/ntd/. Centers for Disease Control and Prevention. Neglected Tropical Diseases.

3. A Situation Analysis: Neglected Tropical Diseases in Bangladesh. *Ministry of Health & Family Welfare, Government of Bangladesh* 2010.

4. http://www.ban.searo.who.int/en/Section3/Section39/Section55.htm. World Health Organization/ SEARO. Surveillance, Prevention and Control of Communicable Disease. Neglected Tropical Disease. .

5. Chowdhury R, Mondal D, Chowdhury V, Faria S, Alvar J, Nabi SG, Boelaert M, Dash AP. How Far Are We from Visceral Leishmaniasis Elimination in Bangladesh? An Assessment of Epidemiological Surveillance Data. *PLoS Negl Trop Dis* 2014. 8(8): e3020.

6. Mahmood B, Mahmood S. Emergence of Dengue in Bangladesh a major international public health concern in recent years. *J Environ Res Manage* 2011. 2(3): 035-041.

7. Ferdousi F, Alam MS, Hossain MS, Ma E, Itoh M, Mondal D, Haque R, Wagatsuma Y. Visceral Leishmaniasis Eradication is a Reality: Data from a Community-based Active Surveillance in Bangladesh. *Trop Med Health* 2012. 40(4): 133-139.

Chapter 2

ROLE OF ACTIVE DISEASE SURVEILLANCE AND NEEM OIL INTERVENTION TO CONTROL VISCERAL LEISHMANIASIS IN BANGLADESH

2.1 SUMMARY

Visceral leishmaniasis (VL), or kala azar, is one of the most neglected tropical diseases in the world, affecting the poorest segments of rural populations. In Bangladesh, more than 20 million people are considered at risk of developing VL. According to the Directorate General of Health Services (DGHS) in Bangladesh, a total of 70 170 VL cases were reported throughout the country during the last 10 years (1999-2010). However, these official figures are thought to represent a gross underestimate. On the other hand, neem (Azadirachta indica A, Juss) as well as some other environment-friendly and easily bio-degradable natural insecticides of plant origin has received much attention for control of medically important arthropods responsible for vector-borne diseases. Therefore, to assess the magnitude of VL and to explore the potential role of neem extract on disease incidence, a community-based intervention study coupled with active disease surveillance was conducted in 8 randomly selected villages in a highly endemic area of Bangladesh from 2006 to 2008.

A total of 6761 individuals living in 1550 mud-walled houses were included in the active surveillance. Rapid rK39 dipstick tests were conducted throughout the study period to facilitate the case diagnosis. Individuals with previous or current clinical leishmaniasis were identified on the basis of the case definition of the VL elimination program. Untreated cases of suspected VL were referred to the hospital for treatment. Socioeconomic and environmental information including bed net use was also collected. In the intervention households (n = 770), 300 ppm diluted neem oil was sprayed bi-weekly during the summer (April to September) and monthly during the remaining

months (October to March) from December 2006 to December 2008. No intervention was carried out for the control households (n = 780).

In 2006, the annual incidence of clinical leishmaniasis in the study area was 141.9 cases per 10 000 population, which was significantly increased by the following year owing to community-based active surveillance for case detection and reporting. However, early case detection and early referral for treatment led to a significant decrease in the incidence rate in 2008. Factors associated with VL included younger age group (3-14 years) (RR = 2.17; 95% CI = 1.39 – 3.37), not having electricity in the household (RR= 2.99; 95% CI= 1.56 – 5.75), never using mosquito-control measures (RR = 1.41; 95% CI = 1.03 – 1.92), and never using a bednet (RR = 1.96; 95% CI = 1.40 – 2.75). Neem oil was not found to be effective in the control of VL; rather, the VL incidence was slightly higher in the intervention area. Probably the result was confounded by some variables, e.g., better socioeconomic condition and higher rate of using mosquito-control measures including bednets in the control area. However, the proportion of increased case reporting in 2007 was significantly higher in control area than in intervention area (z = 5.72; 95% CI = 0.0069 – 0.0139, $p < 0.0001$). The proportion of decreased case reporting in 2008 was significantly higher in intervention area than in control area (z = 2.16; 95% CI = 0.0004 – 0.0133, $p = 0.03$). Therefore, we may assume that neem intervention, along with active disease surveillance, played an important role to decrease the incidence rate of clinical leishmaniasis.

However, massive efforts in community-based active disease surveillance coupled with scaled-up personal protection measures and integrated vector management interventions are required to achieve the goal of the VL elimination program. Neem oil

intervention would be a favorable option for vector management. Further research evidence and innovative application technique is required with the support of local government and international organization.

2.2 INTRODUCTION

2.2.1 Disease Overview

Visceral leishmaniasis (VL) is the most severe form of leishmanises group of disease. The other 2 forms of leishmaniasis are cuteneous leishmaniasis and mucocuteneous leishmaniasis. VL is also called kala-azar (KA; “black fever” in Hindi), “dum-dum fever” or “ponos” (1). It is caused by the intracellular protozoan parasite *Leishmania donovani* and transmitted by female sandfly vectors *Phlebotomus argentipes*. It is one of the most neglected tropical diseases in the world affecting the poorest segments of rural population.

VL is characterized by symptoms such as irregular bout of fever, malaise, weight loss and loss of appetite. Clinical signs include anaemia, wasting, skin darkening, enlargement of spleen, liver and, sometimes lymph nodes. Case-fatality rate of VL is 100% if not treated and nearly 10 % even after treatment (2).

Risk factors for the disease include male gender, young age, selected occupations (e.g. farming), poor economic conditions, malnutrition, and immuno depression (3, 4).

2.2.2 Disease Epidemiology

VL is highly endemic in the Indian subcontinent and in East Africa. An estimated 200 000 to 400 000 new cases of VL occur worldwide each year. Over 90% of new cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan (5). In the Indian subcontinent, it has become a serious public health issue with 500 000 new cases, 60 000 deaths and 1.6 million disability–adjusted life years (DALYs) per year (6, 7).

VL is the main form of the disease in South-East Asia and in the Mediterranean Basin. In South-East Asia, transmission generally occurs in rural areas below 600m above sea level, with a heavy annual rainfall, with a mean humidity above 70%, a temperature range of 15–38 °C, abundant vegetation, subsoil water and alluvial soil. The disease is most common in agricultural villages where houses are frequently constructed with mud walls and earthen floors, and cattle and other livestock live close to humans. In the Mediterranean Basin, VL occurs in rural areas, in villages in mountainous regions and also in some periurban areas, where Leishmania parasites live on dogs and other animals. VL in the Americas is very similar to that found in the Mediterranean Basin. The habit of keeping dogs and other domestic animals inside the house is thought to promote human infection. There are also frequent outbreaks of VL in the northern Acacia–Balanite savanna and the southern savanna and forest areas of East Africa where sandflies live around termite mounds (5).

2.2.3 VL in Bangladesh

VL was first described in 1824, in Jessore district of Bengal which is now Bangladesh. VL appeared to have spread along the courses of the Ganges and Brahmaputra rivers, the major transport routes. In these early outbreaks, the case-fatality rate was reported to be more than 95 per cent. The epidemic that occurred in Jessore from 1824 to 1827 reportedly killed 75 000 people. The first affected village in Dhaka district is said to have disappeared from the map (8, 9).

VL epidemic peaks were recorded in Bengal in the 1820s, 1860s, 1920s, and 1940s. During 1960s VL was almost eliminated in Bangladesh as a mutual effect of malaria eradication program (MEP). The effort was largely based on the widespread indoor residual spraying with DDT. However, a resurgence of the disease occurred during late 1970s when the large scale use of DDT was ceased. There have been sporadic VL cases in the 1970s, and an outbreak occurred in Pabna district in 1980. The districts most affected in the early 1980s were reported to have been Sirajganj, Pabna, Mymensingh, Rajshahi and Tangail (8-11).

At present, 34 out of 64 districts of Bangladesh reported to have VL cases. According to Malaria & Parasitic Disease Control Unit (M&PDC), Directorate General of Health Services of Bangladesh, Mymensingh produces highest number of VL cases during the last 10 years (1999–2008). A total of 70 170 KA cases were reported throughout the country and 41 168 of them (60%) were from Mymensingh district (12).

2.2.4 VL in Japan

Japan is not endemic for VL. All leishmaniasis patients have been imported cases, including over 300 VL, seven PKDL (post kala-azar dermal leishmaniasis), and about 60 cutaneous leishmaniasis cases. In addition, several imported canine leishmaniasis cases have been recently recognized. First case of VL in Japan was reported in 1911. Among other reported VL cases, 218 were soldiers who returned from the People's Republic of China before and after World War II during 1940s (13, 14).

2.2.5 The Vector

There are 500 species of *phlebotomine* species, also known as sandflies, of these about 30 species of the female *Phlebotomous* belonging to six genera are suspected vectors of transmitting parasites. However, *Phlebotomus argentipes* is the only proven vector of VL in Indian subcontinent. They are small (approximately 2–3 mm in length), hairy and soundlessly flying insects. They are found around human habitats and breed in specific organic wastes such as feces, manure, rodent burrows, leaf litter and in dark corners in the crevices of the walls having high humidity and temperature. They are poor flyers and have a flight range of a few kilometers, usually fly quite low and remain in the vicinity of their breeding ground. They are unable to fly in the presence of any wind produced by fan or ventilator also. They require moist soil rich in organic and nitrogenous matter to breed. The larval stages of sandfly present in alluvial or alkaline soil. The damp and dark corners of cattlesheds, where humus is present, and the cracks and crevices in the walls are favourable conditions for *P. argentipes* breeding. The larvae

cannot survive drying out; they will feed on organic waste and then pupate. The female sandfly needs blood in order to obtain the protein necessary to develop its eggs. In its search for blood they cover a radius of a few to several hundred meters around its habitat. They become active from dawn to dusk (15, 16).

2.2.6 Disease Transmission

VL is predominantly transmitted through the bite of an infected female *phlebotomine* sandfly. However, vertical and parenteral transmissions (through blood transfusions and needle sharing) have also been reported (17, 18).

Beside humans, numerous rodent and canine species have been incriminated as reservoirs. Several animal reservoirs have been identified in different countries for leishmaniasis. Female sandflies transmit the parasites from animal to animal, animal to man, and man-to-man. In India, however, the species *Phlebotomous argentipes* transmits the disease from man-to-man (19).

The parasite *Leishmania donovani* has 2 asexual stages of life cycle. In the insect vectors, the parasite is found in a promastigote form which is characterized by elongated, motile and an extracellular stage. In vertebrates the parasite is found in amastigote form. The amastigotes are ovoid, nonmotile and intracellular stage. The vector injects promastigotes into the host's skin and soon after the parasite is taken-up by skin macrophages where the promastigotes transform into amastigote form within 12–24 h of inoculation. After transformation, the amastigotes multiply within the macrophage and ultimately the macrophage bursts releasing the amastigotes to infect other macrophages.

This stage is chronic in nature and may continue for months to years and even for the life time without noticeable signs and symptoms, depending upon the host susceptibility and its immune status. In case of VL, the infected macrophages disseminate to other organs,e.g., liver, spleen, and bone marrow. The amastigotes are taken up by the sandfly after a blood meal. The transformation of amastigotes to promastigotes starts within hours of ingestion. Amastigotes completely transformed into motile promastigotes within 24–48 h and keep on dividing by binary division. The mature metacyclic promastigotes are accumulated in the midgut and foregut of the vector. The sandfly transmit the infection during the another blood meal on the same or another host species (15).

2.2.7 Visceral leishmaniasis elimination program

Availability of a highly sensitive and specific rapid diagnostic test (rK39), an increasing number of treatment options, and the unique anthroponotic features of the sandfly vector make VL a potential candidate for elimination. The unique disease limiting factors of VL in the Indian subcontinent include *Phlebotomus argentipes* as the only vector, humans as the only reservoir, and a defined geographical distribution of the disease. Therefore, a campaign to eliminate VL has been initiated in the indian subcontinent with the support of WHO since 2005.The health ministers of Bangladesh, India and Nepal signed a Memorandum of Understanding for joint efforts to eliminate this deadly disease. The target of the VL elimination program is to reduce the annual incidence of kala azar to less than one per 10 000 at the district or sub district level

(upazila in Bangladesh, sub district in India and district in Nepal) by 2015. Early case detection and treatment together with integrated vector management and health education within the endemic communities are the main strategic pillars of the VL elimination program (20, 21).

Delays in case detection and treatment remain a problem in the control of VL despite some advancement in diagnostics and treatment. The current approach to VL in the region is based on ‘‘passive case detection,’’ i.e., patients are treated if they present themselves to a health care provider. Given the low uptake of health services in this region, the overall effectiveness of the VL elimination program would be maximized if the case detection is organized in a more active manner.

2.2.8 Current vector control strategies

Current vector control strategies are tailored towards reducing the source of the vector by destroying the breeding sites and minimizing the transmission by interrupting vector-man contact.

Residual spraying of houses and cattle shelters with chemical insecticides, e.g., DDT, pyrethroids, malathion, have been demonstrated effective against the *Phlebotomine* vector (22-24). However, the effectiveness of spraying is not the only issue of concern, other problems are the side effects on human health and environment and their sustainability. Several factors such as cost of the insecticides, the logistic constraints low acceptance by the community and low community participation and the emergence of resistance affect the long term effectiveness and sustainability of these interventions (25).

There are also many studies showing that chemical insecticide treated bed-nets (ITN) and curtains are effective measures to control VL (26-29). However, ITN may not reduce the number of female sandfly (30). And also, these chemicals are too expensive for routine public health use, and, like residual spraying, there is a chance to develop resistance in insects.

Some studies also suggest the environmental control of the vector by destroying rodent burrows (31), plastering all the cracks and crevices by mud and lime (32) and constructing cement skirting on the floor (33). For biological control, the information is available only for laboratory settings (34-36); as the application of biolarvicides in the field condition is difficult due to diverse breeding habitat of sandfly and, therefore, their practical application appears to be of limited use in the control of VL. Therefore, a cost-effective, environment-friendly and well-accepted vector control measure for VL is a high priority.

2.2.9 Neem oil for vector control

Neem (*Azadirachta indica* A, Juss) is a tree commonly found in Indian sub-continent and can also grow in most arid sub-tropical and tropical areas of the world (37). In Sanskrit, it is called "cure of all ailments" and has been used for various purposes as “Ayurvedic medicine” since ancient times. Various neem products, such as leaves, twigs, and seed oil have been found to be effective for protecting human from various pathogens and parasites where no side effect was observed (38). In recent years, neem oil as well as some other environment-friendly and easily bio-degradable natural insecticides of plant

origin has received much attention for control of medically important arthropods which are responsible for vector-borne diseases, such as malaria, leishmaniasis, dengue, and filariasis (39-42).

Neem oil is extracted from its seeds and is composed of six complex tetranortriterpenoid limonoids, namely azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin. Their complex chemical structures make them difficult to develop any kind of resistance in insects. Among its compounds, azadirachtin was found the most effective against more than 400 species of insects and mites (40, 43). It acts as repellent, deterrent, anti-feedant and growth regulator rather than directly killing the insects (39-41, 43). Recently, neem oil has been registered as an agrichemical by the Food and Drug Administration (FDA) in the U.S., Ministry of Health, Labor and Welfare in Japan and the Australian Pesticides and Veterinary Medicines Authority (APVMA) in Australia (42).

A number of studies demonstrated the effectiveness of neem limonoids against malaria vector in laboratory settings (41, 44, 45). There are also many studies in field settings with variety of application methods suggesting the effectiveness of neem oil against malaria vector; like applying neem oil mixed with coconut oil to the exposed part of the body (46), burning kerosene lamp with neem oil (47, 48), spraying neem oil-water emulsion (45, 49) or neem extract powder (50) to known breeding habitat. Neem oil was also found effective against filaria vector (*Culex quinquefasciatus; Diptera: Culcidae*) (49, 51) and dengue vector *(Aedes aegypti; Diptera: Culicidae)* (45, 49) in the field settings. Some reports suggest a role for neem oil against the sandfly vector in laboratory settings (52-54), however, there is a little study in field settings.

Neem oil would be a promising option to control VL as well as other vector borne diseases. However, research evidence is required to demonstrate the effectiveness of neem oil against VL.

2.2.10 Purposes of the study

1. To provide information regarding the VL burden in endemic communities of Bangladesh in the early phase of the eradication program and to identify some of the risk factors associated with VL in these areas.
2. To demonstrate the prospect of active surveillance as an important element of the VL elimination program.
3. To evaluate the potential role of azadirachtin (neem extract) to control VL in an endemic area of Bangladesh.

2.3 MATERIALS and METHODS

2.3.1 Study area

Mymensingh is the most endemic district for VL in Bangladesh. Every year, more than 60% of the total VL cases reported in Bangladesh are from this district. The present study was conducted in Trishal, one of the highest VL case-reporting subdistricts of Mymensingh. Trishal consists of 12 unions with an area of 339 km^2 and 80 000 households comprising a total population of 372 000 according to the 2001 census. Two

unions of the Trishal subdistrict were chosen for the study because they had the highest incidence rates of VL according to hospital data in 2003. From these 2 unions, 8 villages were further selected randomly (Figure 2.1). At the beginning of the study total number of mud-walled households in the study villages was enumerated. Area boundary was marked in the area map by using Geographic Information System (GIS). Then households were selected randomly from the study villages. Individual household was considered as a cluster in this study. Households of 3 villages from 1 union were randomly allocated to the intervention group while households of 5 other villages from another union were allocated to the control group. To control dilution of effect intervention and control households were chosen from 2 different unions.

1.3.2 Study design

At the beginning of the study, individuals from each selected household were invited to participate in the community-based active surveillance starting in August 2006. Children aged less than 3 years were excluded from the study because VL is not only difficult to diagnose in small children but is also still uncommon in this age group. Field research assistants completed a household roster and recorded individuals with VL in the household within the previous1 year as per the study case definition. Untreated VL suspected cases were referred to the government hospital for further confirmation and appropriate case management. Information on demographic, socioeconomic, and educational status and mosquito-control measures was also collected.

2.3.3 Neem intervention

In the intervention households, neem oil solution was sprayed from December 2006 to December 2008; while no intervention was carried out for the control households. We used commercially available 300 ppm neem oil (Neem Oil®, Neem Foundation, Dhaka, Bangladesh) and diluted it to 0.5% solution with soap powder (Jet®, P&G, Bangladesh) as an emulsifier (55). Using knapsack sprayers, mud walls were coated with neem oil solution up to the height of 3 meter from the floor. According to national guideline for bio-pesticides, neem oil was sprayed bi-weekly during the summer (April to September) and monthly during the remaining months (October to March).

2.3.4 Active disease surveillance using rapid rK39 dipstick test

To facilitate case management, the rapid rK39 dipstick test was performed for the maximum number of individuals from both intervention and control area available at the beginning of the study (Kalazar Detect®; InBios International, Seattle, WA, USA). According to a recent meta-analysis study, the average sensitivity and specificity of rK39 dipstick test was 92% and 95% respectively (56) . Rapid rK39 dipstick tests were again performed at 1-year intervals for 2 consecutive years (in 2007 and 2008) for suspected VL cases of both intervention and control area. A person with fever $\geq$2 weeks and splenomegaly was considered a 'suspected VL case'. RK39-positive individuals with signs and symptoms of VL were considered as 'probable cases of VL' and referred to the nearby government hospital for further confirmation and treatment. RK39-positive persons without any sign or symptom were considered as 'asymptomatic VL patients'

and advised to contact our health workers who would be working in the villages for the study purposes. For any subsequent complaint of VL symptoms, an additional test was performed and probable VL patients were referred to the government hospital. Field research assistants conducted home visits on holidays and in the early morning and late evening on working days. At least 1 home visit was conducted every month in each village. More frequent home visits, i.e., at least 1 home visit every 2 weeks, were conducted during the summer (April through September). Copies of the treatment record sheets of VL patients were collected from the corresponding government hospital in order to find patients missing from those referred to the hospital by the field assistants. In such cases, field staff visited the missing patients' houses and assisted them in reporting to the hospital.

2.3.5 Study case definition

The following study case definitions were used:

Clinical/confirmed leishmaniasis cases (past cases):

Patient diagnosed with illness characterized by ≥2 weeks of fever and at least one of the following: splenomegaly, skin darkening, and/or weight loss, plus a history of treatment with either sodium stibogluconate or pentamidine with clinical resolution of the symptoms; or with *Leishmania* amastigotes demonstrated in bone marrow or splenic aspirate or tissue in the past one year and documented in the medical records.

Clinical/confirmed leishmaniasis cases (current cases):

Patient diagnosed with illness characterized by ≥2 weeks of fever and at least one of the following: splenomegaly, skin darkening, and/or weight loss; or with *Leishmania* amastigotes demonstrated in relevant aspirate or tissue, and/or a positive rK39 dipstick result.

2.3.6 Statistical analysis

IBM SPSS version 20.0 software was used for the statistical analysis. The general characteristics of the study subjects and households were summarized as the frequency for categorical variables. Age of the study subjects was categorized into 3 groups, 3-14 years, 15-45 years, and >45 years, according to a previous study in Bangladesh which demonstrated high prevalence of VL among younger aged (<45 years) people (57). Comparisons between intervention area and control area were made by chi-square test. The incidence rate of VL was calculated for each year (2006, 2007, and 2008). Rate ratios were computed for the proportions of VL incidence in intervention area and control area. Both univariate and multivariate regression analyses were conducted to determine the association between VL incidence during the intervention (i.e., VL incidence in 2007 and 2008) and neem intervention. Factors which differed significantly between the intervention and control areas (p<0.05) were put in the multivariate models. Restricted factor analysis was conducted to confirm the association. Univariate analysis of the association between VL incidence during the study period (i.e., VL incidence in 2006, 2007, and 2008) and potential risk factors were also conducted. Cochran-Mantel-

Haenszel (CMH) statistics were conducted to measure the relationships between each pair of VL incidence-related variables identified from univariate analysis. A final multivariate model was constructed to determine the relative associations between VL incidence and each significant variable while adjusted for other covariates. Sex was also included in the final multivariate model as evidence was showed in a previous study (58). All the association was determined using a modified Poisson regression analysis with a robust error variance procedure (59). This procedure not only is preferable for the prospective study with binomial outcome but also control the clustering effect, if any. The results were expressed as the relative risk (RR) and 95% confidence interval (CI).

2.3.7 Ethical approval

The protocol was approved by the icddr,b Research and Ethical Review Committee. Informed consent was obtained from all adult participants and from a parent or guardian of all participating children.

2.4 RESULTS

2.4.1 General characteristics of the study participants

There were a total of 1550 mud-walled houses comprising 6955 inhabitants in the study villages. Household interviews were successfully completed for all mud-walled houses. Active disease surveillance was conducted for 6761 individuals aged $\geq$ 3 years in 2006. Table 2.1 shows the individual and household characteristics of the study subjects.

Most of the inhabitants (99.7%) had been living in the study area for more than 3 years. About one thirds (34.7%) were children aged less than 15 years. The average number of household members was 4 (SD = 2) and the average household yearly income was US $526.0 (SD = 333.6). More than 70% of the household heads were illiterate, whereas only 12.2% of them had completed $\geq$5 years of institutional education. Majority of the households (59.5%) owned land and at least one domestic animal (72.9%). Only 23.4% households used some kind of mosquito-control measures; however, most of them (74.1%) were irregular in using those measures. Although 92.1% of the households used bed nets, only 23.8% of them did so regularly.

People living in control area had significantly higher electricity in the households and used more mosquito-control measures and bed nets than the people in intervention area ($p < 0.0001$).

2.4.2 Incidence of clinical leishmaniasis during the study period

Table 2.2 shows the incidence rate of clinical leishmaniasis from 2006 through 2008. In 2006, 96 individuals were identified as having clinical leishmaniasis. In 2007, the number of reported clinical leishmaniasis cases was substantially increased over that of the previous year (RR = 1.38; 95% CI = 1.07 - 1.79). However, the incidence of clinical leishmaniasis in 2008 was significantly lower than in 2006 (RR = 0.19; 95% CI = 0.12 – 0.32).

2.4.3 Outcome of neem intervention

Neem oil solution was sprayed to 770 mud-walled households in the 3 intervention villages which accommodated 3355 inhabitants. On the other hand, there were 780 mud-walled houses comprising 3406 inhabitants in the control villages. Clinical leishmaniasis cases in 2006 were significantly higher in the intervention villages than in the control villages before the neem intervention was started ($p < 0.001$). Even after 1 year of neem intervention in 2007, the number of clinical leishmaniasis cases was significantly higher in the intervention areas than in the control area ($p = 0.01$). After 2 years of neem intervention in 2008, there was no significant difference in clinical leishmaniasis cases between the study areas. Table 2.3 shows that the proportion of increased case reporting in 2007 was significantly higher in control area than in intervention area (Rate ratio = 5.72; 95% CI = 0.0069 – 0.0139). However, the proportion of decreased case reporting in 2008 was significantly higher in intervention area than in control area (Rate ratio = 2.16; 95% CI = 0.0004 – 0.0133).

Table 2.4 shows the results of regression analysis of the association between neem intervention and VL incidence during intervention. Although a significant negative association was found between neem intervention and VL incidence (RR = 1.56; 95% CI = 1.13 – 2.15, $p = 0.01$), no significance existed after adjustment with having electricity in the household, use of mosquito-control measures, and use of bed net (RR = 1.36; 95% CI = 0.98 – 1.89, $p = 0.07$). Restricted factor analysis also confirmed that there was no significant difference in VL cases between the study areas (Table 2.5). The analyses were restricted for who had not electricity in the households, did not use mosquito control measures at night, and did not use bed net at night.

2.4.4 Risk factors associated with clinical leishmaniasis

Table 2.6 shows the results of the Poisson regression analyses for variables significantly associated with clinical leishmaniasis. It was found that VL incidence was significantly different among the 3 age groups (Likelihood Ratio $\chi^2 = 14.78$, df = 2, $p = 0.001$). The younger age group (3-14 years) was 2.17 times more at risk of developing VL than the older age group of >45 years (RR = 2.17; 95% CI = 1.39 – 3.37, $p = 0.001$); but age group of 15-45 years showed borderline significance compared to older age group of >45 years (RR = 1.55; 95% CI = 0.99 – 2.41, $p = 0.055$). Male participants tended to develop VL (4.1%) than did female participants (3.2%); however, this difference was not statistically significant (RR = 1.26; 95% CI = 0.98 – 1.61, $p = 0.066$). People who did not have electricity in the household were at higher risk of developing VL than were people who did have it (RR= 3.40; 95% CI= 1.76 – 6.59, $p < 0.001$). Similarly, people who never used mosquito-control measures or bed nets while sleeping had more risk of developing VL than did those who had at least some habit of using a mosquito-control measure or a bed net ((RR = 1.49; 95% CI = 1.09 – 2.06, $p = 0.013$, and RR = 2.02; 95% CI = 1.44 – 2.84, $p < 0.001$, respectively). The other variables, i.e., education of household head, having own land, having domestic animal, and having cattle shed on the premises, were not found significantly associated with clinical leishmaniasis.

The younger age group (3-14 years) used fewer mosquito-control measures than did the older age group > 45 years (24.8% and 27.6% respectively, $p = 0.082$). Moreover, the male younger age group used fewer mosquito-control measures than did the female younger age group (24.5% and 25.2% respectively, $p = 0.372$). On the other hand, use of

bed net was slightly higher among male participants than the female participants (92.2% and 91.9% respectively, p = 0.695). However, these associations were not found statistically significant. There was also no significant association between use of bed net and use of mosquito-control measures (p =0.068). Significantly positive associations were found between having electricity in the house and using mosquito-control measures ($p < 0.001$), and between having electricity in the house and using bed net ($p < 0.001$).

Table 2.6 shows the result of multivariate Poisson regression analysis of the final model adjusted for all the covariates that have a p value < 0.05 in the univariate analysis. After adjustment, age group of 15-45 years became statistically significant (RR = 1.59; 95% CI = 1.02 – 2.47, p = 0.040). The association of having electricity with clinical leishmaniasis was slightly attenuated, but still remained significant (RR= 2.99; 95% CI= 1.56 – 5.75, p =0.001). The similar result was found for use of mosquito-control measures (RR = 1.41; 95% CI = 1.03 – 1.92, p = 0.031) and use of bed net (RR = 1.96; 95% CI = 1.40 – 2.75, $p < 0.001$).

2.5 DISCUSSIONS

2.5.1 Effect of active disease surveillance

The result of this study suggested that VL might be underreported in 2006 through the existing passive case detection system. The increased incidence rate of clinical leishmaniasis in 2007 may indicate that community-based active surveillance using a simple diagnostic tool (rK39 dipstick test) would be able to substantially increase the case reporting. Early case reporting and referral for treatment could significantly

reduce the source of infection within the community, which resulted in a notably decreased incidence rate of clinical leishmaniasis in 2008. In South Asia as well as in Bangladesh, delays in case detection and treatment remain a problem in the control of VL. The median delay from onset of fever to treatment was reported to be about 4 months (60-62). Moreover, the number of people exposed to infection or infected without any symptom has an important role in disease transmission. Therefore, early diagnosis with active surveillance and early treatment are essential not only to cure the VL patient, but also to decrease the infection reservoir. The rapid rK39 dipstick test has shown high sensitivity and specificity in detecting VL infections in the Indian subcontinent (63). The World Health Organization (WHO) currently recommends it as the best available diagnostic tool for VL for use at health facilities in remote areas. Thus, this kind of simple diagnostic tool may improve active surveillance programs by facilitating case management.

2.5.2 Effect of neem intervention

Neem oil was not found to be effective in the control of VL. During the 1st year of intervention case reporting was 5 times higher in the intervention area than in the control area. On the other hand, during the 2nd year of intervention case reporting decreased dramatically in both intervention and control areas. However, the decreased rate was 2 times higher in the intervention area than in the control area. Therefore, we may assume that neem intervention, along with active disease surveillance, played an important role to decrease the incidence rate of clinical leishmaniasis.

Control villages had better socio-economic condition, and higher rate of using mosquito-control measures including bed nets which are known preventive factors of VL (57, 61). Therefore, we assume that this study suffers from selection (recruitment) bias which might result in underestimate of the effect of neem intervention on VL incidence. Moreover, knowledge of intervention may lead to different intensity of active disease surveillance and VL incidence being diagnosed more often in the intervention area either consciously or subconsciously which might result in detection bias. We used diluted neem oil solution with a low concentration of azadirachtin (300 ppm) to spray the households. A commercially available high concentration of azadirachtin (1500 ppm) was found to be more effective against the sandfly (41). We assumed that the low concentration of azadirachtin used in our study would be more effective as a repellant than environmental use. Another important point would be the application method as only indoor spraying of neem oil was used in this study. Applying neem oil (pure or mixed with coconut oil) to exposed parts of the body (52, 54), burning neem oil in kerosene lamps (47, 62), and spraying neem extract powder on known sandfly breeding habitats (50) have been reported to be effective against many vectors.

Our previous study on people's attitudes toward available neem products has shown that neem oil is a well-accepted method for VL prevention in endemic communities of Bangladesh (64). Neem oil would be a favorable option to control VL in the endemic areas. However, further research evidence and innovative application technique is required with the support of local government and international organization.

2.5.3 Risk factors associated with clinical leishmaniasis

This study found that the children (3-14 years old) of these communities were at more risk of developing VL than the older age group of >45 years, as in other endemic areas of Bangladesh (57). It was also found that male cases of VL were higher than female cases; however, the difference was not statistically significant. A recent study in Nepal found that male participants had a significantly higher risk of developing VL than did female participants (58). Other studies in Bangladesh and India could not find any association between sex and VL incidence (57, 65). Traditionally, men in the countryside keep the upper part of their body exposed and wear fewer clothes than women, particularly in the summer months, which might make them more vulnerable to bites by the sandfly vector. We also found that use of some kind of mosquito-control measures, e.g., smoke and mosquito coil, was protective against VL. However, very few households reported using such measures regularly. Moreover, people in the younger age group, especially young males, used fewer mosquito-control measures than did people in the older age group, which might also make them more susceptible to the sandfly bite.

Previous epidemiologic studies in the Indian subcontinent found bed nets to be a protective factor against VL (57, 60), which is also supported by our findings. More than 90% of households of the study villages reported using bed nets at night. This high percentage suggested that bed nets were already acceptable in Bangladeshi communities. However, only 23.8% of the households reported using bed nets regularly. Previous studies in this subcontinent region demonstrated that insecticide-treated bed nets could be a favorable option for vector management (26, 29). Therefore, health education programs

on personal protection measures followed by provision of insecticide-treated bed nets might be a highly effective prevention intervention in such an endemic community.

VL is known as a disease of the poorest of the poor in the Indian subcontinent (66). Although determining the relationship between poverty and VL involves multiple factors (67), poverty has been found to be associated with VL in previous studies conducted in this region (57, 61). In the present study, having electricity in the household, which may constitute an indicator of better socioeconomic status, was found to confer less risk of developing VL. Previous studies in Nepal (58, 60) and Bangladesh (57) found that ownership of cattle was strongly protective against VL. Another study in India found that illiteracy was associated with VL risk (65). However, in this study, neither cattle ownership nor illiteracy of the household head was associated with VL incidence in the univariate analysis and thus was not included in the multivariate analysis.

This study was not designed for comprehensive risk factor analysis. Therefore, we could not report some of the important factors of recent interest, such as immunogenetic factors, dietary indicators, and nutritional status, which might play important role to influence the susceptibility of a host to the development of VL infection (57, 68).

2.6 CONCLUSION

Availability of a highly sensitive and specific rapid diagnostic test (rK39), an increasing number of treatment options, and the unique anthroponotic features of the sandfly vector make VL a potential candidate for elimination. However, massive efforts in community-based active disease surveillance coupled with scaled-up personal

protection measures and integrated vector management interventions are required to achieve the goal of the VL elimination program.

Neem oil would be a favorable option as an environment-friendly, well accepted and cost effective measure to control VL among the marginalized poor of the endemic areas. However, further research evidence and innovative application technique is required with the support of local government and international organization.

2.7 REFERENCES

1. Modabber F. Tropical Disease Research: A Global Partnership; ed. John Maurice and Anna Marina Pearce; World Health Organization, Geneva. 1987. 99-112.

2. Bora D. Epidemiology of visceral leishmaniasis in India. *Natl Med J India* 1999. 12(2): 62-68.

3. Desjeux P. Leishmaniasis. Public health aspects and control. *Clin Dermatol* 1996. 14(5): 417-423.

4. Dye C, Williams BG. Malnutrition, age and the risk of parasitic disease: visceral leishmaniasis revisited. *Proc Biol Sci* 1993. 254(1339): 33-39.

5. http://www.who.int/mediacentre/factsheets/fs375/en/. World Health Organization. Leishmaniasis. Fact sheet N°375. January, 2014.

6. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004. 27(5): 305-318.

7. Murray CJL, Lopez AD. ed. Global health statistics: A compendium of incidence, prevalence, and mortality estimates for over 200 conditions, Vol. II. Boston: Harvard University Press. 1996.

8. Sengupta PC. History of kala-azar in India. *Indian Med Gaz* 1947. 82: 281-286.

9. Sanyal RK. Leishmaniasis in the Indian sub-continent. In: Chang KP, Bray RS, eds. Leishmaniasis. Amsterdam: Elsevier Science Publishers, B.V., 1985. 443-467.

10. Birley MH. An historical review of malaria, kala-azar and filariasis in Bangladesh in relation to the Flood Action Plan. *Ann Trop Med Parasitol* 1993. 87(4): 319-334.

11. Elias M, Rahman AJ, Khan NI. Visceral leishmaniasis and its control in Bangladesh. *Bull World Health Organ* 1989. 67(1): 43-49.

12. Alam MS, Wagatsuma Y, Mondal D, Khanum H, Haque R. Relationship between sand fly fauna and kala-azar endemicity in Bangladesh. *Acta Trop* 2009. 112(1): 23-25.

13. Endo S. A case of Kala-Azar in Japan. *Tokyo Igakkai Zasshi (in Japanese)* 1911. 25: 473.

14. Takada S, Otomo H, Izeki M, Kimata I. Imported parasitic diseases in Japan. in: Report of the Research on Chemotherapy for Tropical Diseases, Japanese Ministry of Health and Welfare, Tokyo. 1990. p181.

15. Sharma U, Singh S. Insect vectors of Leishmania: distribution, physiology and their control. *J Vector Borne Dis* 2008. 45(4): 255-272.
16. Swaminath CS, Short HE, Anderson LAP. Transmission of Indian kala-azar to man by the bite of P. argentipes. *Indian J Med Res* 1942. 30: 473-477.

17. Mescouto-Borges MR, Maues E, Costa DL, Pranchevicius MC, Romero GA. Congenitally transmitted visceral leishmaniasis: report of two Brazilian human cases. *Braz J Infect Dis* 2013. 17(2): 263-266.

18. Martin-Sanchez J, Pineda JA, Morillas-Marquez F, Garcia-Garcia JA, Acedo C, Macias J. Detection of Leishmania infantum kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other Leishmania infection markers. *Am J Trop Med Hyg* 2004. 70(5): 545-548.

19. Singh S. New developments in diagnosis of leishmaniasis. *Indian J Med Res* 2006. 123(3): 311-330.

20. Regional strategic framework for elimination of kalaazar from the South-East Asia region (2005-2015) New Delhi: Regional Office for South-East Asia SEA-VBC-85 (Rev-1). *World Health Organization* 2005. WHO.

21. http://www.who.int/tdr/news/2011/vl-elimination/en/. Eliminating visceral leishmaniasis: A multi-pronged approach. Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization. 2011.

22. Joshi RD, Rai RN. Impact of DDT spraying on populations of P. argentipes and P. papatasi in Varanasi district, Uttar Pradesh. *J Commun Dis* 1994. 26(1): 56-58.

23. Kaul SM, Sharma RS, Dey KP, Rai RN, Verghese T. Impact of DDT indoor residual spraying on *Phlebotomus argentipes* in a kala-azar endemic village in eastern Uttar Pradesh. *Bull World Health Organ* 1994. 72(1): 79-81.

24. Das ML, Roy L, Rijal S, Paudel IS, Picado A, Kroeger A, Petzold M, Davies C, Boelaert M. Comparative study of kala-azar vector control measures in eastern Nepal. *Acta Trop* 2010. 113(2): 162-166.

25. Kishore K, Kumar V, Kesari S, Dinesh DS, Kumar AJ, Das P, Bhattacharya SK. Vector control in leishmaniasis. *Indian J Med Res* 2006. 123(3): 467-472.

26. Picado A, Das ML, Kumar V, Kesari S, Dinesh DS, Roy L, Rijal S, Das P, Rowland M, Sundar S, Coosemans M, Boelaert M, Davies CR. Effect of village-wide use of long-lasting insecticidal nets on visceral Leishmaniasis vectors in India and Nepal: a cluster randomized trial. *PLoS Negl Trop Dis* 2010. 4(1): e587.

27. Elnaiem DA, Elnahas AM, Aboud MA. Protective efficacy of lambdacyhalothrin-impregnated bednets against Phlebotomus orientalis, the vector of visceral leishmaniasis in Sudan. *Med Vet Entomol* 1999. 13(3): 310-314.
28. Elnaiem DA, Aboud MA, El Mubarek SG, Hassan HK, Ward RD. Impact of pyrethroid-impregnated curtains on Phlebotomus papatasi sandflies indoors at Khartoum, Sudan. *Med Vet Entomol* 1999. 13(2): 191-197.

29. Mondal D, Chowdhury R, Huda MM, Maheswary NP, Akther S, Petzold M, Kumar V, Das ML, Gurung CK, Ghosh D, Kroeger A. Insecticide-treated bed nets in rural Bangladesh: their potential role in the visceral leishmaniasis elimination programme. *Trop Med Int Health* 2010. 15(11): 1382-1389.

30. Dinesh DS, Das P, Picado A, Davies C, Speybroeck N, Ostyn B, Boelaert M, Coosemans M. Long-lasting insecticidal nets fail at household level to reduce abundance of sandfly vector *Phlebotomus argentipes* in treated houses in Bihar (India). *Trop Med Int Health* 2008. 13(7): 953-958.

31. Vyokov VN. Control of sandflies. Gamaleya Institute of Epidemiology and Microbiology. *In: WHO Travelling Seminar on Leishmaniasis Control. Moscow* 1980. 6-16.

32. Kumar V, Kesari SK, Sinha NK, Palit A, Ranjan A, Kishore K, Saran R, Kar SK. Field trial of an ecological approach for the control of *Phlebotomus argentipes* using mud & lime plaster. *Indian J Med Res* 1995. 101: 154-156.

33. Dhiman RC. Effect of minor engineering intervention in the control of breeding of Phlebotomus papatasi (Scopoli) sandflies. *Southeast Asian J Trop Med Public Health* 1995. 26(2): 368-370.

34. De Barjac H, Larget I, Killick-Kendrick R. [Toxicity of Bacillus thuringiensis var. israelensis serotype HI4, for larvae of sandflies, the leishmaniasis vectors (author's transl)]. *Bull Soc Pathol Exot Filiales* 1981. 74(5): 485-489.

35. Robert LL, Perich MJ, Schlein Y, Jacobson RL, Wirtz RA, Lawyer PG, Githure JI. Phlebotomine sand fly control using bait-fed adults to carry the larvicide Bacillus sphaericus to the larval habitat. *J Am Mosq Control Assoc* 1997. 13(2): 140-144.

36. Robert LL, Perich MJ, Schlein Y, Jacobson JL. Bacillus sphaericus inhibits hatching of phlebotomine sand fly eggs. *J Am Mosq Control Assoc* 1998. 14(3): 351-352.

37. Copping LG. The BioPesticides Manual; A world compendium of naturally occurring biopesticides (Eds.): Second Edition. British Crop Protection Counsel, UK; 2001.

38. Bostid FRR. Neem: a tree for solving global problems: report of an ad hoc panel of the board of Science and technology for international development, National Research Council: National academy press, Washington DC, USA; 1992.

39. Mordue (Luntz) AJ, Nisbet AJ. Azadirachtin from the Neem Tree *Azadirachta indica*: its Action Against Insects. *An Soc Entomol Brasil* 2000. 29(4): 615-632.

40. Schmutterer H. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annu Rev Entomol* 1990. 35: 271-297.

41. Nathan SS, Kalaivani K, Murugan K. Effects of neem limonoids on the malaria vector Anopheles stephensi Liston (Diptera: Culicidae). *Acta Trop* 2005. 96(1): 47-55.

42. Wagatsuma Y, Alam MS, Fukushige M, Islam MZ, Itoh M, Mondal D, Haque R. Neem Extract as a Control Tool for Vector-borne Diseases: An Example of Visceral Leishmaniasis in Bangladesh. *Biopestic Int* 2009. 5(3): 134-140.

43. Mordue (Luntz) AJ, Blackwell A. Azadirachtin: an Update. *J Insect Physiol* 1993. 39(11): 903-924.

44. Awad OM, Shimaila A. Operational use of neem oil as an alternative anopheline larvicide. Part A: Laboratory and field efficacy. *East Mediterr Health J* 2003. 9(4): 637-645.

45. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (Azadirachta indica) formulation against mosquitoes. *Malar J* 2009. 8: 124.

46. Sharma VP, Ansari MA, Razdan RK. Mosquito repellent action of neem (Azadirachta indica) oil. *J Am Mosq Control Assoc* 1993. 9(3): 359-360.

47. Sharma VP, Ansari MA. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. *J Med Entomol* 1994. 31(3): 505-507.

48. Ansari MA, Razdan RK. Operational feasibility of malaria control by burning neem oil in kerosene lamp in Beel Akbarpur village, District Ghaziabad, India. *Indian J Malariol* 1996. 33(2): 81-87.

49. Batra CP, Mittal PK, Adak T, Sharma VP. Efficacy of neem oil-water emulsion against mosquito immatures. *Indian J Malariol* 1998. 35(1): 15-21.

50. Gianotti RL, Bomblies A, Dafalla M, Issa-Arzika I, Duchemin JB, Eltahir EA. Efficacy of local neem extracts for sustainable malaria vector control in an African village. *Malar J* 2008. 7: 138.

51. Ravindran J, Eapen A, Kar I. Evaluation of repellent action of neem oil against the filarial vector, Culex quinquefasciatus (Diptera: Culicidae). *Indian J Malariol* 2002. 39(1-2): 13-17.

52. Sharma VP, Dhiman RC. Neem oil as a sand fly (Diptera: Psychodidae) repellent. *J Am Mosq Control Assoc* 1993. 9(3): 364-366.

53. Srinivasan R, Kalyanasundaram M. Relative efficacy of DEPA and neem oil for repellent activity against Phlebotomus papatasi, the vector of leishmaniasis. *J Commun Dis* 2001. 33(3): 180-184.

54. Kebede Y, Gebre-Michael T, Balkew M. Laboratory and field evaluation of neem (Azadirachta indica A. Juss) and Chinaberry (Melia azedarach L.) oils as repellents against Phlebotomus orientalis and P. bergeroti (Diptera: Psychodidae) in Ethiopia. *Acta Trop* 2010. 113(2): 145-150.

55. Wagatsuma Y, Dhar I, Alam MS, Khanum H, Wahed MA, Haque R. Neem oil as biological control against Phlebotomine sand fly. *Trop Med Health* 2006. 33(suppl): 72-76.

56. Maia Z, Lirio M, Mistro S, Mendes CM, Mehta SR, Badaro R. Comparative study of rK39 Leishmania antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. *PLoS Negl Trop Dis* 2012. 6(1): e1484.

57. Bern C, Hightower AW, Chowdhury R, Ali M, Amann J, Wagatsuma Y, Haque R, Kurkjian K, Vaz LE, Begum M, Akter T, Cetre-Sossah CB, Ahluwalia IB, Dotson E, Secor WE, Breiman RF, Maguire JH. Risk factors for kala-azar in Bangladesh. *Emerg Infect Dis* 2005. 11(5): 655-662.

58. Rijal S, Uranw S, Chappuis F, Picado A, Khanal B, Paudel IS, Andersen EW, Meheus F, Ostyn B, Das ML, Davies C, Boelaert M. Epidemiology of Leishmania donovani infection in high-transmission foci in Nepal. *Trop Med Int Health* 2010. 15 Suppl 2: 21-28.

59. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004. 159(7): 702-706.

60. Bern C, Joshi AB, Jha SN, Das ML, Hightower A, Thakur GD, Bista MB. Factors associated with visceral leishmaniasis in Nepal: bed-net use is strongly protective. *Am J Trop Med Hyg* 2000. 63(3-4): 184-188.

61. Thakur CP. Socio-economics of visceral leishmaniasis in Bihar (India). *Trans R Soc Trop Med Hyg* 2000. 94(2): 156-157.

62. Ahluwalia IB, Bern C, Costa C, Akter T, Chowdhury R, Ali M, Alam D, Kenah E, Amann J, Islam M, Wagatsuma Y, Haque R, Breiman RF, Maguire JH. Visceral

leishmaniasis: consequences of a neglected disease in a Bangladeshi community. *Am J Trop Med Hyg* 2003. 69(6): 624-628.

63. Sundar S, Singh RK, Bimal SK, Gidwani K, Mishra A, Maurya R, Singh SK, Manandhar KD, Boelaert M, Rai M. Comparative evaluation of parasitology and serological tests in the diagnosis of visceral leishmaniasis in India: a phase III diagnostic accuracy study. *Trop Med Int Health* 2007. 12(2): 284-289.

64. Fukushige M, Alam MS, Haque R, Wagatsuma Y. Acceptance for Neem Oil as a Visceral Leishmaniasis Vector Control Tool in Bangladesh. *Biopestic Int* 2009. 5(2): 141–147.

65. Ranjan A, Sur D, Singh VP, Siddique NA, Manna B, Lal CS, Sinha PK, Kishore K, Bhattacharya SK. Risk factors for Indian kala-azar. *Am J Trop Med Hyg* 2005. 73(1): 74-78.

66. Boelaert M, Meheus F, Sanchez A, Singh SP, Vanlerberghe V, Picado A, Meessen B, Sundar S. The poorest of the poor: a poverty appraisal of households affected by visceral leishmaniasis in Bihar, India. *Trop Med Int Health* 2009. 14(6): 639-644.

67. Alvar J, Yactayo S, Bern C. Leishmaniasis and poverty. *Trends Parasitol* 2006. 22(12): 552-557.

68. Karplus TM, Jeronimo SM, Chang H, Helms BK, Burns TL, Murray JC, Mitchell AA, Pugh EW, Braz RF, Bezerra FL, Wilson ME. Association between the tumor necrosis factor locus and the clinical outcome of Leishmania chagasi infection. *Infect Immun* 2002. 70(12): 6919-6925.

Chapter 3

IDENTIFICATION AND MANAGEMENT OF KEY CONTAINER FOR *AEDES* LARVAL BREEDING TO CONTROL DENGUE IN BANGLADESH

3.1 SUMMARY

Dengue fever (DF), one of the most important emerging arboviral diseases worldwide, is transmitted through the bite of container breeding mosquitoes *Aedes aegypti* and *Aedes albopictus*. In Bangladesh, DF has become a serious public health concern after the first large-scale outbreak in 2000. Since then, DF cases have been reported every year in all major cities of Bangladesh.

To identify important breeding habitats of *Aedes* mosquitoes, a household entomological survey was conducted in Dhaka from August through October 2000, the peak epidemic period of DF/DHF. Approximately 100 households (range 100-119) were randomly selected from each of the 90 administrative wards in Dhaka City Corporation. All water holding containers were inspected for *Aedes* larvae in all 3 locations of each household, i.e., indoor, outdoor, and rooftop.

Of 9,222 households inspected, 1,306 households (14.2%) were found positive for *Aedes* larvae. Of 38 777 wet containers were examined, 2216 wet containers (5.7%) were found infested with *Aedes* larvae. The overall house index (HI), breteau intex (BI) and container index (CI) were 14.2, 24.6 and 5.9 respectively. Positive wet containers were significantly higher in number in outdoor than in indoor and in rooftop. Among the positive containers, the most commons were earthen jars (19.9%), flower pots (16.2%), tires (14.9%), drums (9.8%), tanks (9.1%), and cans and bottles (8.2%). A total of 3 027 867 *Aedes* larvae were collected, among which 1 923 648 (63.5%) were *Aedes aegypti*. Number of *Aedes aegypti* was higher than the number of *Aedes albopictus* in all 3

locations (92.7:1, 1.4:1, and 9.9:1 in indoor, outdoor, and rooftop locations respectively). Independent type of household, having water storage system in the household, and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae.

The study results would reinforce the dengue vector control strategy to have focus on the containers that are consistent producers of *Aedes* larvae and houses that consistently have *Aedes* larvae in containers.

3.2 INTRODUCTION

3.2.1 Disease overview

DF is an acute febrile disease caused by one of four closely related, but antigenically distinct, dengue virus serotypes designated as DEN-1, DEN-2, DEN-3, and DEN-4 of the genus Flavivirus. Infection with one of these serotypes provides lifelong immunity against that serotype, but it does not provide cross-protective immunity against the other three. There is evidence that sequential infection increases the risk of the more severe disease, Dengue Hemorrhagic fever (DHF). Persons living in a dengue-endemic area can have up to four dengue infections, thereby putting them at risk for DHF with each subsequent infection.

DF may present as a mild febrile syndrome similar to the flu, an undifferentiated febrile illness with a maculopapular rash (often seen in children), or the classical disease with two or more of the following manifestations: fever, headache, bone or joint pain,

muscular pain, rash, pain behind the eyes, hemorrhagic manifestations (e.g., petechiae). During dengue epidemics, hemorrhagic complications may also appear, such as bleeding from the gums, nosebleeds, and bruising. There is no specific treatment for DF beyond symptomatic treatment, rest, and rehydration.

DHF is characterized by four clinical manifestations, all of which must be present: (1) fever or recent history of acute fever, (2) hemorrhagic phenomena (presence of at least one of the following: positive tourniquet test; petechiae, ecchymoses, or purpura; or bleeding from mucosa, gastrointestinal tract, injection sites, or others), (3) thrombocytopenia (100 000 mm^3 or less), and (4) plasma leakage due to increased capillary permeability. Moderate to marked thrombocytopenia with concurrent hemoconcentration is a distinctive clinical laboratory finding of DHF. However, the major pathophysiological change that determines the severity of disease in DHF, and differentiates it from DF, is plasma leakage manifested by a rising hematocrit value (i.e., hemoconcentration). It is very important to distinguish between DF with hemorrhagic symptoms and DHF so that appropriate therapy can be initiated in the case of DHF. Case fatality due to DF is very low, but case fatality due to DHF can be high.

Dengue shock syndrome (DSS) is the most severe form of DHF, and is characterized by the presence of all four DHF clinical manifestations as well as circulatory failure. All three manifestations of circulatory failure must be present: rapid and weak pulse; narrow pulse pressure or hypotension; and cold, clammy skin and altered mental state (1-3).

3.2.2 Global burden of dengue

DF is one of the most important emerging diseases which have become a serious public health concern. It is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. Over 2.5 billion people, around 40% of the world's population, are now at risk of dengue. It is estimated that there may be 50–100 million dengue infections worldwide every year.

DHF was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Only 9 countries had experienced DHF before 1970, however, the number had increased more than 4-fold by 1995. An estimated 500 000 people with DHF require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die.

Recently the number of reported cases has continued to increase as the disease spreads to new areas. Cases across the Americas, South-east Asia and Western Pacific have exceeded 1.2 million cases in 2008 and over 2.3 million in 2010. In 2013, 2.35 million cases of dengue were reported in the Americas alone, of which 37 687 cases were DHF (3).

3.2.3 Dengue in Bangladesh

In Bangladesh, the first documented outbreak of dengue occurred in 1964 which was known as 'Dhaka fever'. There were few scattered cases of DF during 1977-1978 and 1996-1997 (4, 5). However, DF has become a serious public health threat in Bangladesh after the first large-scale outbreak in 2000 with 5551 cases. Among the reported cases 4385 (62.4%) were dengue fever infections and 1186 (37.6%) cases were dengue hemorrhagic fever. The case fatality rate (CFR) was 1.7% with 93 deaths reported. Since 2000, DF cases have been reported every year in all major cities of Bangladesh. The worst outbreak was in 2002 with 6104 cases and 58 deaths. In 2005 there were 1048 reported cases and 4 deaths. In 2006 the number of cases and deaths increased by 2 fold as compared to 2005. In 2010, 5500 people were infected, with 98 deaths (6).

3.2.4 Dengue in Japan

In Japan, endemic dengue cases had been reported in Okinawa since 1893 (7). There were dengue outbreaks in Japan from 1942 to 1945. It was first emerged in Nagasaki in August 1942 and soon spread to other cities such as Sasebo, Hiroshima, Kobe, and Osaka, recurring every summer until 1945 (8). However, domestic outbreaks have not been reported since 1945. But there have been many imported dengue cases (9). A total of 406 cases of imported dengue virus infection were confirmed from 2003 to 2010. However, this year Japan is battling its first outbreak of dengue fever in almost 70

years. The Ministry of Health, Labor and Welfare confirmed that the number of reported dengue fever cases stood at 81 in 15 prefectures as of September 09, 2014.

In Japan, DF and dengue haemorrhagic fever (DHF) have been Category IV notifiable infectious diseases regulated by the Infectious Disease Control Law of Japan since April 1999. Physicians in all clinics and hospitals are required to report demographic information and clinical and exposure history about every patient meeting the DF/DHF case definitions to the nearby public health centre. The data are reported by local governments to the Ministry of Health, Labour and Welfare and the Infectious Disease Surveillance Center, National Institute of Infectious Diseases.

3.2.5 Disease Transmission

Dengue viruses are transmitted to humans through the bites of infective female *Aedes* mosquitoes. Mosquitoes generally acquire the virus while feeding on the blood of an infected person. After virus incubation for 4 - 10 days, an infected mosquito is capable of transmitting the virus to susceptible individuals for the rest of its life. Infected female mosquitoes may also transmit the virus to their offspring by transovarial (via the eggs) transmission.

Infected humans are the main carriers and amplifying hosts of the virus. However, some studies have shown that in some parts of the world monkeys may become infected and perhaps serve as a source of virus for uninfected mosquitoes. The virus circulates in the blood of infected humans for 2 - 7 days, at approximately the same time as they have

fever. *Aedes* mosquitoes may acquire the virus when they feed on an individual during this period (3).

3.2.6 The Vector

The *Aedes aegypti* mosquito is the primary vector of dengue. *Ae. aegypti* is a small, dark mosquito with white lyre shaped markings and banded legs. It lives in urban habitats and is closely associated with humans and their dwellings. People not only provide the mosquitoes with blood meals but also water-holding containers in and around the home needed to complete their development. The mosquito lays her eggs on the sides of containers with water and eggs hatch into larvae after a rain or flooding. A larva changes into a pupa in about a week and into a mosquito in two days. *Ae. aegypti* is extremely common in areas lacking piped water systems, and depend greatly on water storage containers to lay their eggs. Male and female adults feed on nectar of plants; however, female mosquitoes need blood in order to produce eggs, and are active in the daytime. It breeds mostly in artificial containers, but has been reported in natural containers as well. Artificial water containers may include water storage containers, flower pots, discarded tires, plates under potted plants, cemetery vases, flower pots, buckets, tin cans, clogged rain gutters, ornamental fountains, drums, water bowls for pets, birdbaths. This species has also been found in underground collections of water such as open or unsealed septic tanks, storm drains, wells, and water meters. Some natural habitats are like tree holes, leaf-axils, etc. *Ae. aegypti* bites primarily during the day. This species is most active for approximately two hours after sunrise and several hours before

sunset, but it can bite at night in well lit areas. Female *Ae. aegypti* bites multiple people during each feeding period (10).

Ae. albopictus is a secondary dengue vector in Asia. *Ae. albopictus* is a small, dark mosquito with a white dorsal stripe and banded legs. It is also called 'Asian tiger mosquito'. It lays its eggs on the inner sides of water-holding receptacles in urban, suburban, and rural areas as well as in nearby edges of forested areas. *Ae. albopictus* is closely associated with vegetated areas in and around homes. The immature forms (larvae and pupae) are found in artificial containers with water. Larvae can also be found in natural habitats such as tree holes, rock holes, hollow bamboo stumps, and leaf axils. *Ae. albopictus* is a very aggressive daytime biter. Its peak feeding times are during the early morning and late afternoon. They bite outdoors and indoors, but are usually found outside. *Ae. albopictus* is highly adaptive and therefore can survive in cooler temperate regions of Europe. Its spread is due to its tolerance to temperatures below freezing, hibernation, and ability to shelter in microhabitats (11).

Both of the vectors became more widespread following uncontrolled urbanization in the second half of the 20th century. Sparse vegetation, low altitude, good transportation routes, and urban development favor the transmission of vectors (12).

3.2.7 Immunization

There is no vaccine to protect against dengue. Developing a vaccine against DF or DHF has been challenging for a number of reasons. With 4 closely related viruses that can cause the disease, the vaccine must immunize against all 4 types to be effective.

There is limited understanding of how the disease typically behaves and how the virus interacts with the immune system (3). However, recent studies find the quasispecies of dengue virus. Therefore, these viruses may frequently develop a resistance to vaccines because of their high mutation rates. Dengue virus quasispecies found in mosquitoes and some substitutions potentially change the conformation of envelope protein, indicating the possible escape from the host immune system. If this is the case, it may be difficult to develop an effective dengue virus vaccine because a vaccine against limited strains may be unable to fight a diverse dengue virus population. There is also lack of laboratory animal models available to test immune responses to potential vaccine (13).

3.2.8 Current Prevention and Control Strategies

There is no vaccine against dengue and there are no drugs to treat DHF and DSS. Hence, vector control remains the cornerstone for the prevention and control of dengue (14). Dichlorodiphenyltrichloroethane (DDT) was one of the first chemical control measures used to target adult stages of the dengue vector. Significant reductions in vector populations were achieved, but the development of DDT resistance was one of the key factors that led to the re-emergence of dengue from the 1960s onward (12). Meanwhile, second- and third generation insecticides became available (eg malathion and pyrethroids). However, chemical control of dengue vectors has shortcomings, including environmental contamination, bioaccumulation of toxins and concerns regarding human toxicity, which are especially linked to the use of insecticides in drinking- water containers (15).

Alternative methods consist of biological control (e.g. the introduction of larvivorous organisms such as fish, copepods and insect larvae into water containers), the release of transgenic vectors (aimed at reducing or even replacing the wild-type vector population with one that has a reduced capacity to transmit and reproduce), and environmental management. Environmental management provides a flexible framework through which a wide variety of actions can be undertaken in an integrated and coherent fashion, such as source reduction of the vectors, provision of safe water, covering and screening of water containers, and reduction of human – vector contact by screening doors and windows, and using insecticide-treated nets. Social mobilization, a process to obtain and maintain the involvement of various groups and sectors of the community in the control of disease and/or its vector has traditionally been used to engender community participation in broad-based dengue prevention and control activities such as community clean-up campaigns, physical management of containers, use of chemical and biological control methods, improved environmental management at the community levels, and education to recognize DHF signs and symptoms. Integrated control measures have also been 17developed usually facilitated through community-based approaches (12, 16, 17).

3.2.9 Key Container

Until a vaccine, clinical cure, or genetic strategy is available, control of dengue will continue to depend on suppression of the vector populations or interference of the vector-human interaction (18). It is, however, a futile exercise to keep on killing mosquitoes in the presence of an almost unlimited number of breeding sites, for the larvae laid at these sites soon grow into adult mosquitoes (19). For the same reason, generalized community clean-up campaigns of vector breeding sites have had only a transient and limited effect, if at all, on disease incidence. Theoretically, the identification and subsequent elimination of the most *Aedes* mosquito producing containers in a given area may potentially reduce mosquito density below a critical threshold, which could result in more efficient and cost-effective control campaigns (20, 21).

In most areas there are a relatively small number of containers that consistently serve as the primary producers of *Aedes* larvae, with other containers playing minor roles in mosquito production. "Key containers" are these primary source adult *Aedes* mosquitoes (17). The contribution of a container class to the vector population depends on the productivity of that specific class and its abundance. Productivity is determined by survival and developmental rate of larvae and pupae (22), which depends on a wide range of abiotic and biotic factors, such as temperature, physical shape, material with which the container is made, use and source of water in the container, size of the container, location, availability of resources (i.e., food), and competition among co specifics (23-29). Consequently, each ecological setting has its own unique set of key containers (21, 30). For example, in Mexico tires and bottles were the most important contributors to the *Ae. aegypti* population (31), in Vietnam large concrete tanks and water storage jars were

the main source of immature *Ae. aegypti* development (32), whereas in Peru 57% of adult *Ae. aegypti* production originated from outdoor-unlidded containers that were passively filled with rainwater (29). In Brazil, the most productive container types were water tanks, metal drums, and kitchen items (33).

A "key container" survey for improved dengue vector surveillance and vector control was developed (1994-1997) and implemented on a regional basis in 1997 in Vietnam. This program was selected as one of the 'best practices for environmental management of dengue' by USAID in 2003 (17). By focusing on the containers that are consistent producers of larvae and houses that consistently have *Aedes* larvae in containers, control measures can be tailored for the specific needs of the area and populace. Once the most productive key containers are identified, targeted control of dengue vectors becomes more affordable and feasible. At the same time, targeted vector control can help minimize the use of chemicals that may be costly and have other long-term health and environment impacts.

3.2.10 Objective of the study

The aim of this study was to identify the containers which served as primary producers of *Aedes* larvae during the deugue outbreak in Bangladesh. In most areas there are a relatively small number of containers that consistently serve as the primary breeding sites of *Aedes* larvae. The contribution of a container category to the vector population depends on the productivity of that specific container category and its abundance. Therefore, abundance and mosquito productivity of each container type were determined

in order to propose specific and effective vector control messages specific. This study also aimed to identify some risk factors for the households to be infested with *Aedes* larvae.

3.3 MATERIALS and METHODS

3.3.1 Study area

A household entomological survey was conducted in Dhaka from August through October 2000, the peak epidemic period of DF/DHF. Dhaka city is situated between $23^0$52'49" N to $23^0$41'12" N latitude and $90^0$20'09" E to $90^0$27'04" E longitudes. Dhaka encompasses 347 km^2 of area with an estimated population of 15.4 million. This study was conducted within the Dhaka Municipality area, formerly named as Dhaka City Corporation (DCC). DCC was divided into 90 smallest administrative units called ward. According to 2001 population census, DCC had 1 107 000 households, and a total population of 5 378 000. Bangladesh has a tropical monsoon-type climate, with a hot and rainy summer and a pronounced dry season in the cooler months. Dhaka meets all the criteria for rapid breeding of *Aedes* mosquito as the temperature and large rainfall with rapid urbanization and dense population (34).

3.3.2 Household survey

For field survey, approximately 100 households (range 100-119) were randomly selected from each of the 90 wards according to the proportional distribution of house structure types. Household types were categorized as independent house, multi-storey house, semi-permanent house, slum, and others. Household was defined as one separate unit of accommodation, and the immediately surrounding premises. Field survey was conducted by 46 teams comprising of 2 field research assistants in each team. The team interviewed the household head or other adult resident according to a pre-tested structured questionnaire to collect information on socio-demography, awareness on dengue and its vector control, and self-reprting dengue cases. Field research assistants also looked for containers with standing water, and for *Aedes* larvae within the containers. All 3 locations of each household, i.e., indoor, outdoor, and rooftop, were inspected for potential wet containers. Possibly all larvae, that could not be identified in the field, were collected in labeled specimen bottles, and were reared up to the adult stage to identify species. Before the field survey, field research assistants were trained on inspecting wet containers, collecting and identifying larvae, and recording data. The indices for *Aedes* larval population were calculated to determine the distribution and density of the vector.

House index (HI) = percentage of houses positive for *Aedes* larvae

Container index (CI) = percentage of wet containers positive for *Aedes* larvae

Breteau index (BI) = number of positive containers for *Aedes* larvae per 100 houses inspected

3.3.3 Wet container categorization

Total 111 types of wet containers were found with the maximum 76 types in outdoor location of the households. The containers were then categorized into 11 different groups- flower pots, buckets, water tanks, drums, tires, discarded appliances, plastic bowls, earthen pots, coconut shells, cans and bottles, and others. All unusual and less abundant container types that eventually were found positive were classified as 'others', such as ant guard, air conditioner drip pan, refrigerator drip pan, polythene bag, bath tub, tree hole, bamboo stump, and leaf axil. Although buckets, water tanks, drums, plastic bowls, and earthen jars mostly had common purpose of use, i.e., water storage, we opted to keep all the varieties instead of a common category to have a detailed profile view of wet containers served as potential breeding habitats of *Aedes* larvae.

3.3.4 Statistical analysis

A descriptive analysis was done for the distribution of wet containers and *Aedes* larvae along 3 locations. Firstly, number of different wet containers in 3 locations was listed to identify the most abundant container categories in different locations. Secondly, percentage of each container category was calculated to identify their larval productivity. Finally, the contribution of each container category to total positive containers was calculated. The relative frequency of each container category as an *Aedes* larval breeding site in different locations was featured as two-dimensional presentation (35). Slope =1 is considered as the equality line. If the containers were equally utilized as breeding sites, all points fall on the equality line. If the percentage of positivity of any container category

exceeds the percentage of contribution to total wet containers (slope>1), the point for the container falls above the equality line. This container is then considered to be an essential container for *Aedes* larval breeding. Conversely, less importance is indicated for the container having slope of <1 (i.e., if the point falls below the equality line).

Univariate logistic regression analysis was conducted to determine the risk factors associated with household infestation with *Aedes* larvae. The significance level was set at $p < 0.05$. However, variables with a p value < 0.1 (Wald Chi-square test) in the univariate analysis were selected to include in the multivariate model. The purpose was to identify variables which, by themselves, were not significantly related to household infestion of *Aedes* larvae but would make an important contribution in the presence of other variables. IBM SPSS version 20.0 software was used for the statistical analysis.

3.3.5 Ethical approval

Permission to carry out this study was provided by the of icddr,b Research and Ethical Review Committee. Signed informed consent was obtained from each household that participated in the study.

3.4 RESULTS

3.4.1 Summary of the entomological survey

The results of the entomological survey are summarized in Table 1. Of 9222 households inspected, 1306 households (14.2%) were found positive for *Aedes* larvae. Multi-storey houses were the highest in number (39.6%) followed by semi-permanent houses (30.4%) and independent houses (20.5%). There were 771 slum houses (8.4%). Household positivity rate was the highest in independent houses (18.6%) followed by slum houses (14.3%), semi-permanent houses (12.9%), and multi-storey houses (12.8%). Of 38,777 wet containers were examined, 2216 wet containers (5.7%) were found infested with *Aedes* larvae. Number of wet containers was abundant in outdoor (56.5%) followed by indoor (32.2%), and rooftop (11.3%). More than two thirds of the positive containers were found in outdoor (77.4%). Among the outdoor containers, 7.8% containers were found infested with *Aedes* larvae. Among the indoor and rooftop containers, 3.1% and 3.9% containers were found positive respectively.

The indices for *Aedes* larval population are listed in Table 2. The overall HI was 14.2. BI was 24.6 and CI was 5.9. All of the indices were in high level of risk for dengue transmission (1).

3.4.2 Key Containers in different locations

Figure 3.2a shows the number of each container category inspected in 3 locations. Among the wet containers, buckets were the most abundant (n = 6580) followed by

flower pots (n = 6066), cans and bottles (n = 5034), and earthen jars (n = 5018). Other water reserving containers, such as drums (n = 2945), and tanks (n = 2675) were also high in number. Buckets (29.5%), flower pots (19.4%), and drums (11.8%) were common among indoor wet containers, while earthen jars (15.2%), cans and bottles (14.9%), and miscellaneous wet containers (14.6%) were common among outdoor wet containers. Among rooftop wet containers, flower pots (33.7%), cans and bottles (18.2%), earthen jars (11.7%), and tanks (10.1%) were common. Among the water reserving containers, buckets were more common in indoor (56.0%) than in outdoor (39.6%). Similarly, drums were more abundant in indoor (50.3%) than in outdoor (42.0%). On the other hand, most of the earthen jars (66.5%) and tanks (61.5%) were found in outdoor. Most of the coconut shells (98.6%), Tires (78.9%), discarded appliances (76.9%), cans and bottles (64.9%), and miscellaneous wet containers (63.4%) were also found in outdoor.

Figure 3.2b shows the percentage of each container category infested with *Aedes* larvae. Among the tires inspected, 27.9% were found positive for *Aedes* larvae of which 23% were in outdoor. The next three highly positive containers were earthen jars (9.0%), tanks (7.7%), and drums (7.6%), all of which were used as water reservoirs. Earthen jars in outdoor were more prone to be positive (7.9%) than those are in indoor (0.7%). Similarly, tanks and drums in outdoor were more positive (4.4% and 4.6% respectively) than in indoor (2.9% and 2.4% respectively). Although buckets were the most abundant, only 1.8% of buckets were found infested with *Aedes* larvae.

Figure 3.2c depicts the percentage contribution of each container category to total positive containers. Of the 2 216 positive containers, the most commons were earthen jars

(19.9%), flower pots (16.2%), tires (14.9%), drums (9.8%), tanks (9.1%), and cans and bottles (8.2%).

3.4.3 Two-dimensional presentation for essential containers

Among indoor containers, tanks were found to be the most essential container for *Aedes* larvae breeding (Figure 3.3a). Tanks constituted only 4.7% of all wet containers but accounted for 20.8% of all positive containers in indoor. Similarly, drums and flower pots constituted 11.8% and 19.4% of all wet containers respectively but accounted for 18.2% and 22.4% of all positive containers in indoor. Therefore, drums and flower pots may also be considered as essential containers in indoor. On the other hand, buckets represented 29.5% of all indoor containers but accounted for only 7.3% of all indoor positive containers. Therefore, buckets fall below the equality line and less importance is indicated for them.

Tires constituted only 4.4% of all outdoor containers but accounted for 16.2% of all outdoor positive containers. Earthen jars represented 15.2% and 23.3% of all outdoor containers and all outdoor positive containers respectively. Similarly, flower pots and drums constituted 9.9% and 5.7% of all wet containers respectively but accounted for 15.2% and 7.9% of all positive containers in outdoor. Therefore, tires, earthen jars, flower pots, and drums can be considered as essential containers in outdoor (Figure 3.3b). Buckets in outdoor, same as in indoor, were found to be less important for *Aedes* larvae breeding. Buckets accounted for only 4.3% of all outdoor positive containers in spite of

representing 11.9% of all outdoor containers. Among the outdoor containers, less importance is indicated for tanks, and cans and bottles also (Figure 3.3b).

Tires and drums were found to be the most important containers in rooftop (Figure 3.3c). Tires constituted 3.7% and 29.3% of all rooftop containers and all positive containers respectively. Drums constituted 5.2% of all rooftop containers but accounted for 9.8% of all rooftop positive containers. Buckets represented 6.5% of all rooftop containers but accounted for 7.5% of all rooftop positive containers. Therefore, buckets in rooftop were found to be borderline essential containers. Flower pots represented 33.7% of all rooftop containers, however, these constituted only 13.2% of all rooftop positive containers. Therefore, flower pots in indoor and in outdoor exhibited more importance as *Aedes* larval breeding sites than flower pots in rooftop sites.

Figure 3.3d shows that overall tires, earthen jars, flower pots, tanks, drums, and plastic bowls were found to be essential containers for *Aedes* larval breeding. Less importance is indicated for buckets, cans and bottles, and discarded appliances.

3.4.4 *Aedes* larval population

Figure 3.4 shows the number of both *Ae. aegypti* and *Ae. albopictus* by 3 locations, i.e., indoor, outdoor, and rooftop (in logarithm scale). A total of 3 027 867 *Aedes* larvae were collected, among which 1 923 648 (63.5%) were *Ae. aegypti*. The density of *Ae. aegypti* was higher in outdoor (81.4%) compared to other 2 locations. The ratio of the total number of *Ae. aegypti* larvae in 3 locations were 8 : 39.6 : 1 (indoor : outdoor : rooftop). *Ae. albopictus* also had higher density in outdoor (0.9 : 276.7 : 1).

About 99% of *Ae. albopictus* were found in outdoor. The number of *Ae. aegypti* was higher than the number of *Ae. albopictus* in all 3 locations (92.7 : 1, 1.4 : 1, and 9.9 :1 in indoor, outdoor, and rooftop respectively).

Table 3 shows the *Aedes* larval productivity of the wet containers. Tanks showed the highest productivity for *Ae. aegypti* larvae both in indoor (80.2%) and in outdoor (46.1%). In rooftop, more than 70% *Ae. aegypti* larvae were found in buckets. Overall around 50% of *Ae. aegypti* were found in tanks, among which 37.5% were in outdoor tanks. All 4 water reservoirs, i.e., tanks, earthen jars, buckets, and drums contained around 90% of *Ae. aegypti* larvae, among which around 72% were found in outdoor. Overall tires contained only 2.7% of *Ae. aegypti* . For *Ae. albopictus* larvae, flower pots (35.6%) and tanks (33.5%) showed the high productivity in indoor. Earthen jars and flower pots were the highest productive containers for *Ae. albopictus* in outdoor (86.2%) and in rooftop (72.9%) respectively. Overall earthen jars alone contained about 86% of *Ae. albopictus*, almost all of them were found in outdoor. Tanks contained 2.9% of *Ae. albopictus* larvae. Other 2 water reservoirs, drums and buckets, did not constitute much at the larval productivity (0.5% and 0.2% respectively). Tires contained 3.5% of *Ae. albopictus.*

3.4.5 Factors associated with household infestion of *Aedes* larvae

Table 4 shows the result of logistic regression analysis for the factors significantly related to household infestion of *Aedes* larvae. Factors which were found significant in the univariate analysis were put in multivariate model. Multivariate logistic regression analysis shows that independent household (OR = 1.57; 95% CI = 1.35 – 1.83, $p < 0.001$), having any kind of water storage system (i.e., tanks, drums, earthen jars, and buckets) in the household (OR = 1.55; 95% CI = 1.33 – 1.82, $p < 0.001$), and having fully/partly shaded outdoor premise (OR = 1.51; 95% CI = 1.34 – 1.70, $p < 0.001$) were significantly associated with household infestation of *Aedes* larvae. 'Used mosquito spray /coil /smoke', and 'Used insecticide during the last 1 month' were not found significant in the univariate analysis.

3.5 DISCUSSIONS

3.5.1 Key containers in different locations

Our analyses revealed that water storage containers, such as earthen jars, tanks, and drums were consistently more likely to contain *Aedes* larvae similar as other studies (18, 33). In indoor, tanks and drums were the most productive; while in outdoor earthen jars were the most productive. Drums were highly productive in rooftop. Although present in abundant, buckets did not constitute much in larval production. Understanding the cultural traditions of owning and using containers is important to identify the key containers in different locations. Dhaka city has a scarcity of domestic water supply and

90% of the municipal water supply is mainly derived from groundwater. Most of the city dwellers store water from supplied pipe water. They either send the pipe water directly to the rooftop tanks or store in the underground reservoirs and pump it to the rooftop tanks. Underground reservoirs are categorized here as outdoor tanks. Tanks in outdoor and rooftop are normally kept covered and closed; therefore, these reservoirs are protected from mosquitoes. As the municipal water supply is not guaranteed all the time, people use to store water in drums, earthen jars, buckets, and in indoor tanks for further use of water. Buckets are relatively smaller in size compared to other water storage containers and are frequently used for washing clothes, cleaning house, and transferring water from one place to another. These practices would reduce the chances of breeding of larvae in buckets. However, apparently unattractive or frequently cleaned containers, if present in large numbers, may still serve as breeding sites for a large portion of the *Aedes* population. On the other hand, drums, earthen jars, and in indoor tanks are bigger in size than buckets and contain large volume of water. Water in these containers is never emptied and is replenished periodically. Moreover, containers in outdoor and in rooftop are not always covered, sometimes letting them unintentionally collect rainwater, and therefore making them the perennial breeding sites of *Aedes* mosquitoes.

Another important breeding site was the tires. Around 28% of tires were found infested with *Aedes* larvae. They constituted 15% of all positive containers and consistently contain *Aedes* larvae in all 3 locations. Usually tires are left abandoned. The collected rain water in tires is an ideal source of *Aedes* larvae (31).

3.5.2 *Aedes* larval population

This study finds that both *Ae. aegypti* and *Ae. albopictus* breeded in indoor and outdoor similar as previous study (36, 37). However, number of *Ae. aegypti* was 2 times higher than the number of *Ae. albopictus*. Moreover, *Ae. aegypti* was found to be the dominant indoor breeder, while *Ae. albopictus* showed higher affinity for outdoor containers. Previous studies on the habitation of *Aedes* mosquitoes showed that *Ae. albopictus* usually seems to be restricted to wooded areas next to humans. Conversely, *Ae. aegypti* can be found in a variety of urban habitats including the highly urbanized areas without wooded vegetation (38). Additionally, *Ae. aegypti* depends highly on human blood and tends to bite and rest indoors, whereas *Ae. albopictus* feeds on a variety of vertebrates outdoors (39). Therefore, *Ae. aegypti* predominates in highly urbanized areas, specially indoor containers. *Ae. albopictus* predominates in rural areas, and in outdoor containers. It seems that *Ae. aegypti* is better adapted than *Ae. albopictus* to the environment of crowded tropical cities like Dhaka. Our study finds that indoor tanks were the highest productive containers (80%) for *Ae. aegypti* and outdoor earthen jars were the highest productive containers (86%) for *Ae. albopictus*. Although highest percentage of tires was found positive, they did not contain much number of *Aedes* larvae. One possible cause would be they contained less amount of water compared to the water storage containers. However, in previous studies, containers with unintentionally collected rainwater were more likely to be infested than other potential developmental sites (28, 40). In Peru, it was estimated that 57% of adult *Ae. aegypti* production could be eliminated by treating outdoor, unlidded containers that were passively filled with rainwater (29).

3.5.3 Factors associated with household infestion of *Aedes* larvae

We have found that independent households, having water storage system in the household and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae. Usually independent households have larger space compared to other types of households and also have both underground and rooftop water reservoir tanks. Latter 2 factors can easily be explained as they provide suitable environment for *Aedes* larval breeding.

3.5.4 Possible preventive measures

Our study results suggest that elimination of the water storage containers and tires would possibly reduce the major portion of the *Aedes* larval population density.

Phunanukoonnon et al. suggested that one preventive measure related to lifestyle is weekly cleaning of containers (41). Similarly, Arunachalam et al. reported that a lack of use of the container for the 7 or more days had a strong positive association with the number of pupae found in household containers (42). Another study in rio de janeiro also found that open-mouthed and large containers are the most suitable for larval production (33). Therefore, community-based educational programs aiming to train householders to use water containers appropriately, such as sealing of containers with lids or nets, cleaning indoor water storage containers regularly, and disposing unused containers, would be favorable intervention program to reduce the larval breeding sites. Applying some biological agents to the water storage containers may also be an effective control tool for vector density as they are usually cheap, and can be maintained by householders

with minimal training (43). Using mesocyclopes in Laos (44), Mexico (45), and Colombia (46) were found effective vector control intervention. Some studies also found that using larvivorus fish can also be an effective biological control tool (41, 47). However, biological control interventions need to be locally adapted and should take into account cultural practices relating to water storage and the social acceptability of keeping living organisms in storage containers of drinking water. For tires, disposal of unused tires would be the best possible intervention. However, using lime to tires was also found to be effective to reduce vector breeding in discarded tires (17).

Therefore, integrated vector management, including community-based health education program, and environmental management, would be sustainable effort for dengue control and prevention.

3.6 CONCLUSION

This study revealed that water storage containers, such as earthen jars, tanks, and drums were consistently more likely to contain *Aedes* larvae. Another important breeding site was the abandoned tires. *Ae. aegypti* was found to be the dominant indoor breeder. *Ae. albopictus* showed higher affinity for outdoor containers compared to indoor containers. We have also found that independent households, having water storage system in the household and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae.

Until a vaccine, clinical cure, or genetic strategy is available, control of dengue will continue to depend on suppression of the vector populations or interference of the vector- human interaction. Generalized community clean-up campaigns of vector breeding sites have had only a transient and limited effect, if at all, on disease incidence. The identification and subsequent elimination of the most *Aedes* mosquito producing containers in a given area may potentially reduce mosquito density below a critical threshold, which could result in more efficient and cost-effective control campaigns.

3.6 REFERENCES

1. http://www.paho.org/english/hcp/hct/vbd/dengue.htm. Pan American Health Organization. 1994. Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control. Scientific Publication No. 548 (in English and Spanish). Washington, D.C.: PAHO.

2. http://www.cdc.gov/Dengue/. Centers for Disease Control and Prevention.

3. http://www.who.int/mediacentre/factsheets/fs117/en/#. World Health Organization. Dengue and severe dengue. Fact sheet N°117, March 2014.

4. Russell PK, Buescher EL, McCown JM, Ordonez J. Recovery of dengue viruses from patients during epidemics in Puerto Rico and East Pakistan. *Am J Trop Med Hyg* 1966. 15(4): 573-579.

5. Amin MMM, Hussain AMZ, Nahar K, Chowdhury IA, Murshed M, Chowdhury SA. Sero-diagnosis of dengue infections in four metropolitan cities of Bangladesh. *Dengue Bull* 2000. 24: 29-33.

6. Mahmood B, Mahmood S. Emergence of Dengue in Bangladesh a major international public health concern in recent years. *J Environ Res Manage* 2011. 2(3): 035-041.

7. Tadano M, Okuno Y, Fukunaga T, Fukai K. Retrospective serological studies on dengue epidemics in Osaka and Okinawa. *Biken J* 1983. 26(4): 165-167.

8. Hotta S. Twenty years of laboratory experience with dengue virus. In: Saunders M and Lennette EH. *eds. Medical and Applied Virology. St. Louis; Geen* 1965. 228–256.

9. Takasaki T. Imported dengue fever/dengue hemorrhagic fever cases in Japan. *Trop Med Health* 2011. 39(4 Suppl): 13-15.

10. http://www.cdc.gov/dengue/resources/30Jan2012/aegyptifactsheet.pdf. Centers for Disease Control and Prevention. Entomology and Ecology. Dengue and the *Aedes aegypti* mosquito.

11. http://www.cdc.gov/dengue/resources/30Jan2012/albopictusfactsheet.pdf. Centers for Disease Control and Prevention. Entomology and Ecology. Dengue and the *Aedes albopictus* mosquito.

12. Dengue Haemorrhagic Fever: Diagnosis, Treatment, prevention and Control. *Geneva: World Health Organization* 1997.

13. Kurosu T. Quasispecies of dengue virus. *Trop Med Health* 2011. 39(4 Suppl): 29-36.

14. Kay B. Dengue vector surveillance and control. *Curr Opin Infect Dis* 1999. 12(5): 425-432.

15. Curtis CF, Lines JD. Should DDT be banned by international treaty? *Parasitol Today* 2000. 16(3): 119-121.

16. Heintze C, Velasco Garrido M, Kroeger A. What do community-based dengue control programmes achieve? A systematic review of published evaluations. *Trans R Soc Trop Med Hyg* 2007. 101(4): 317-325.

17. Lloyd LS. Best Practices for Dengue Prevention and Control in the Americas. Environmental Health Project. 2003. Strategic report 7.

18. Koenraadt CJ, Jones JW, Sithiprasasna R, Scott TW. Standardizing container classification for immature *Aedes aegypti* surveillance in Kamphaeng Phet, Thailand. *J Med Entomol* 2007. 44(6): 938-944.

19. Ko YC, Chen MJ, Yeh SM. The predisposing and protective factors against dengue virus transmission by mosquito vector. *Am J Epidemiol* 1992. 136(2): 214-220.

20. Focks DA, Chadee DD. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. *Am J Trop Med Hyg* 1997. 56(2): 159-167.

21. Tun-Lin W, Kay BH, Barnes A. Understanding productivity, a key to *Aedes aegypti* surveillance. *Am J Trop Med Hyg* 1995. 53(6): 595-601.

22. Southwood TR, Murdie G, Yasuno M, Tonn RJ, Reader PM. Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand. *Bull World Health Organ* 1972. 46(2): 211-226.

23. Barbosa P, Peters MT, Greenough NC. Overcrowding of mosquito populations: responses of larval *Aedes aegypti* to stress. *Environmental Entomology* 1972. 1: 89-93.

24. Moore CG, Whitacre DM. Competition in Mosquitos .2. Production of *Aedes*-Aegypti Diptera-Culicidae Larval Growth Retardant at Various Densities and Nutrition Levels. *Annals of the Entomological Society of America* 1972. 65(4): 915-&.

25. Gilpin ME, McClelland GA. Systems analysis of the yellow fever mosquito *Aedes aegypti*. *Fortschr Zool* 1979. 25(2-3): 355-388.

26. Focks DA, Sackett SR, Bailey DL, Dame DA. Observations on container-breeding mosquitoes in New Orleans, Louisiana, with an estimate of the population density of *Aedes aegypti* (L.). *Am J Trop Med Hyg* 1981. 30(6): 1329-1335.

27. Dye C. Competition amongst larval *Aedes aegypti*. *Ecol Entomol* 1984. 9: 355-357.

28. Strickman D, Kittayapong P. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *Am J Trop Med Hyg* 2003. 68(2): 209-217.

29. Morrison AC, Gray K, Getis A, Astete H, Sihuincha M, Focks D, Watts D, Stancil JD, Olson JG, Blair P, Scott TW. Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. *J Med Entomol* 2004. 41(6): 1123-1142.

30. Tun-Lin W, Kay BH, Barnes A. The Premise Condition Index: a tool for streamlining surveys of *Aedes aegypti*. *Am J Trop Med Hyg* 1995. 53(6): 591-594.

31. Lloyd LS, Winch P, Ortega-Canto J, Kendall C. Results of a community-based *Aedes aegypti* control program in Merida, Yucatan, Mexico. *Am J Trop Med Hyg* 1992. 46(6): 635-642.

32. Kay BH, Nam VS, Tien TV, Yen NT, Phong TV, Diep VT, Ninh TU, Bektas A, Aaskov JG. Control of *Aedes* vectors of dengue in three provinces of Vietnam by use of Mesocyclops (Copepoda) and community-based methods validated by entomologic, clinical, and serological surveillance. *Am J Trop Med Hyg* 2002. 66(1): 40-48.

33. Maciel-de-Freitas R, Marques WA, Peres RC, Cunha SP, de Oliveira RL. Variation in *Aedes aegypti* (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. *Mem Inst Oswaldo Cruz* 2007. 102(4): 489-496.

34. Hossain MI, Wagatsuma Y, Chowdhury MA, Ahmed TU, Uddin MA, Nazmul Sohel SM, Kittayapong P. Analysis of some Socio-demographic Factors Related to DF/DHF Outbreak in Dhaka City. *Dengue Bulletin* 2000. 24: 34-41.

35. Moore CG, Cline BL, Ruiz-Tiben E, Lee D, Romney-Joseph H, Rivera-Correa E. *Aedes aegypti* in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. *Am J Trop Med Hyg* 1978. 27(6): 1225-1231.

36. Lee HL, Cheong WH. A preliminary *Aedes aegypti* larval survey in the suburbs of Kuala Lumpur city *Trop Biomed* 1987. 4: 111-118.

37. Lee HL. A nationwide resurvey of the factors affecting the breeding of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) in urban towns of Peninsular Malaysia 1988-1999. *Trop Biomed* 1991. (8): 157-160.

38. Chan KL, Ho BC, Chan YC. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 2. Larval habitats. *Bull World Health Organ* 1971. 44(5): 629-633.

39. Teng HJ, Wu YL, Lin TH. Mosquito fauna in water-holding containers with emphasis on dengue vectors (Diptera: Culicidae) in Chungho, Taipei County, Taiwan. *J Med Entomol* 1999. 36(4): 468-472.

40. Kittayapong P, Strickman D. Distribution of container-inhabiting *Aedes* larvae (Diptera: Culicidae) at a dengue focus in Thailand. *J Med Entomol* 1993. 30(3): 601-606.

41. Phuanukoonnon S, Mueller I, Bryan JH. Effectiveness of dengue control practices in household water containers in Northeast Thailand. *Trop Med Int Health* 2005. 10(8): 755-763.

42. Arunachalam N, Tana S, Espino F, Kittayapong P, Abeyewickreme W, Wai KT, Tyagi BK, Kroeger A, Sommerfeld J, Petzold M. Eco-bio-social determinants of dengue vector breeding: a multicountry study in urban and periurban Asia. *Bull World Health Organ* 2010. 88(3): 173-184.

43. Kay B, Vu SN. New strategy against *Aedes aegypti* in Vietnam. *Lancet* 2005. 365(9459): 613-617.

44. Jennings CD, Phommasack B, Sourignadeth B, Kay BH. *Aedes aegypti* control in the Lao People's Democratic Republic, with reference to copepods. *Am J Trop Med Hyg* 1995. 53(4): 324-330.

45. Gorrochotegui-Escalante N, Fernandez-Salas I, Gomez-Dantes H. Field evaluation of Mesocyclops longisetus (Copepoda: Cyclopoidea) for the control of larval *Aedes aegypti* (Diptera Culicidae) in northeastern Mexico. *J Med Entomol* 1998. 35(5): 699-703.

46. Suarez-Rubio M, Suarez ME. The use of the copepod Mesocyclops longisetus as a biological control agent for *Aedes aegypti* in Cali, Colombia. *J Am Mosq Control Assoc* 2004. 20(4): 401-404.

47. Martínez-Ibarra JA, Guillén YG, Arredondo-Jiménez JI, Rodríguez-López MH. Indigenous fish species for the control of *Aedes aegypti* in water storage tanks in southern Mexico. *BioControl* 2002. 47: 481-486.

Chapter 4

CONCLUSION

NTDs are a group of infectious diseases affecting more than 1 billion people worldwide; mostly those living in remote rural areas, urban slums or conflict zones. Beyond their negative impact on health, NTDs contribute to an ongoing cycle of poverty and stigma. The global health community and the international organizations infer that control of NTDs represent the opportunity to alleviate poverty in the world's poorest populations, with a direct impact on the achievement of the Millennium Development Goals. NTDs have become a public health burden in Bangladesh as a large number of people is affected each year by these communicable diseases. Marginalized population and the poorest of the poor, often with limited accessibility to health care, are more affected by these diseases. This necessitates effective and sustainable public health intervention strategies to reduce and eliminate the burden caused by these diseases.

In my study, I have analyzed 2 large scale studies on visceral leishmaniasis and dengue, two of the most prevalent NTDs in Bangladesh, in quest of effective and sustainable control strategies.

The study on VL shows that community-based active surveillance using a simple diagnostic tool (rK39 dipstick test) would be able to substantially increase the case reporting. Early case reporting and referral for treatment could significantly reduce the source of infection within the community, which resulted in a notably decreased incidence rate of clinical leishmaniasis. Some personal protection measures, such as using mosquito control measures (dhup, mosquito coil), and using bed net can significantly reduce the chance of getting infected by preventing vector-man contact. Neem oil intervention was not found directly effective to control VL, however proportion analysis shows that the proportion of clinical leishmaniasis case reporting was

significantly decreased in the neem intervention area. Therefore, we may assume that neem intervention, along with active disease surveillance, played an important role to decrease the incidence rate of clinical leishmaniasis. Neem oil would be a favorable option as an environment-friendly, well accepted and cost effective measure to control VL among the marginalized poor of the endemic areas. However, further research evidence and innovative application technique is required with the support of local government and international organization.

Study on dengue larval habitats revealed that water storage containers, such as earthen jars, tanks, and drums were consistently more likely to contain *Aedes* larvae. Another important breeding site was the abandoned tires. *Ae. aegypti* was found to be the dominant indoor breeder. *Ae. albopictus* showed higher affinity for outdoor containers compared to indoor containers. We have also found that independent households, having water storage system in the household and having fully/partly shaded outdoor premise were significantly associated with household infestation of *Aedes* larvae. Until a vaccine, clinical cure, or genetic strategy is available, control of dengue will continue to depend on suppression of the vector populations or interference of the vector- human interaction. It is, however, a futile exercise to keep on killing mosquitoes in the presence of an almost unlimited number of breeding sites, for the larvae laid at these sites soon grow into adult mosquitoes. For the same reason, generalized community clean-up campaigns of vector breeding sites have had only a transient and limited effect, if at all, on disease incidence. Once the most productive key containers are identified, targeted control of dengue vectors becomes more affordable and feasible. At the same time, targeted vector control

can help minimize the use of chemicals that may be costly and have other long-term health and environment impacts.

I hope that the study results would reinforce the NTDs elimination program by focusing on the specific needs and targeted control measures effective for each NTD. In future, I would like to continue my research on NTDs to propose a compact vector control measure for the vector-borne NTDs in Bangladesh.

TABLES & FIGURES

Table 2.1: Characteristics of the study subjects (n = 6761) and households (n = 1550)

	No.	%	Intervention area (n = 3355)	Control area (n = 3406)	*P*-value
Age, years (n = 6761)					
3-14	2344	34.7	1158	1186	0.16
15-45	3365	49.8	1647	1718	
>45	1052	15.6	550	502	
Sex (n = 6761)					
Male	3429	50.7	1675	1754	0.19
Female	3332	49.3	1680	1652	
Education of household head, years (n = 1550)					
0	1129	72.9	572	557	0.33
1-5	232	15.0	113	119	
>5	189	12.2	85	104	
Have own land (n = 1550)	923	59.5	461	462	0.79
Have electricity in the house (n = 1550)	168	10.8	38	130	<0.0001
Share a bedroom with others (n = 1550)	1110	71.6	557	553	0.53
Have domestic animals (n = 1550)	1130	72.9	543	587	0.04
Have a cattle shed on the premises (n = 1550)	671	43.3	322	349	0.24
Use mosquito-control measures at night (n = 1550)	363	23.4	143	220	<0.0001
Mosquito coil	131	36.1	69	62	<0.0001
Smoke (burning straw etc.)	232	63.9	74	158	
Frequency of use of mosquito-control measures (n = 363)					
Always	62	17.1	22	40	0.001
Sometimes	269	74.1	118	151	
Only in summer	32	8.8	3	29	
Use bed net at night (n=1550)	1428	92.1	681	747	<0.0001
Frequency of bed-net use (n=1428)					
Always	340	23.8	209	131	<0.0001
Sometimes	953	66.7	437	512	
Only in summer	135	9.5	33	102	

Table 2.2: Incidence of clinical leishmaniasis in the study area

	No.	Incidence (per 10,000)	Relative risk (RR) (95% CI)
Clinical leishmaniasis in 2006	96	141.9	reference
Clinical leishmaniasis in 2007	133	196.7	1.38* (1.07-1.79)
Clinical leishmaniasis in 2008	19	28.1	0.19** (0.12-0.32)

*$p < 0.05$

**$p < 0.001$

Table 2.3: Comparison of proportions of clinical leishmaniasis cases between intervention and control area

	Intervention area (n = 3355)	Control area (n = 3406)	*P*-value	Rate Ratio (95% CI)
in 2006 (before intervention)	79	17	<0.001	5.72 (0.0069- 0.0139)
one year after intervention	80	53	0.01	2.16 (0.0004-0.0133)
two years after intervention	12	07	0.24	

Table 2.4: Regression models for the association between neem intervention and clinical leishmaniasis cases during the intervention

Regression models	Relative Risk (RR)	95% CI	*P*-value
Neem intervention only	1.56	1.13-2.18	0.01
Neem intervention, Use mosquito-control measures at night	1.49	1.08-2.08	0.02
Neem intervention, Use bed net at night	1.54	1.10-2.14	0.01
Neem intervention, Have electricity in the house	1.46	1.04-2.03	0.03
All variables above	1.36	0.98-1.92	0.07

Table 2.5: Restricted factor analysis for clinical lesihmaniasis cases in the intervention areas

	Intervention area Cases/ Total	Control area Cases/ Total	Unadjusted RR (95% CI)	*P*-value	Adjusted RR (95% CI)	*P*-value
Did not use any mosquito-control measures at night	76/2705	51/2358	1.31 (0.91-1.87)	0.14	1.18[†] (0.82-1.69)	0.38
Did not use bed net at night	17/402	0/137	--	0.01	--	
Did not have electricity in the house	89/3170	56/2809	1.42 (1.01-1.99)	0.04	1.31[‡] (0.93-1.85)	0.012

[†] Adjusted by use of bed net at night and had electricity in the households

[‡] Adjusted by use of any mosquito-control measures at night and use of bed net at night

Table 2.6: Factors related to clinical leishmaniasis

	Clinical leishmaniasis No. of cases	Univariate analysis[†]	Multivariate analysis[‡]
		RR (95% CI)	RR (95% CI)
Age, y			
3-14	111	2.17 (1.39-3.37)**	2.17 (1.39-3.37)**
15-45	114	1.55 (0.99-2.41)	1.59 (1.02-2.47)*
>45	23	reference	reference
Sex			
Male	140	1.26 (0.98-1.61)	1.26 (0.99-1.62)
Female	108	reference	reference
Have electricity in the house			
No	239	3.40 (1.76-6.59)***	2.99 (1.56-5.75)**
Yes	9	reference	reference
Use mosquito-control measures at night			
Never	203	1.49 (1.09-2.06)*	1.41 (1.03-1.92)*
Always/sometimes	45	reference	reference
Use bed net at night			
Never	37	2.02 (1.44-2.84)***	1.96 (1.40-2.75)***
Always/sometimes	211	reference	reference

[†]Univariate Poisson regression analysis

[‡]Multivariate Poisson regression analysis adjusted for age, sex, having electricity in the house, use of mosquito-control measures at night, and use of bed nets at night

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

Table 3.1: Summary of the entomological survey

	Number inspected	Number positive	Percentage
House types	**9222**	**1306**	**14.2**
Independent houses	1890	352	18.6
Multi-storey houses	3651	466	12.8
Semi-permanent houses	2801	364	12.9
Slum houses	771	110	14.3
Others	109	14	12.8
Number of Wet Containers by location	**38,777**	**2272**	**5.7**
Indoor	12,499	384	3.1
Outdoor	21,902	1714	7.8
Rooftop	4376	174	3.9

Table 3.2: *Aedes* Larval population indices

Indices	
House index (HI)	14.2
Breteau index (BI)	24.6
Container index (CI)	5.9

Table 3.3: Container productivity for *Aedes* larvae in different locations

	Aedes aegypti			*Aedes* **albopictus**			
	Total larvae	% of total larvae	Cumulative % of total larvae		Total larvae	% of total larvae	Cumulative % of total larvae
Indoor				**Indoor**			
Tank	255161	80.2	80.2	Flower pot	1222	35.6	35.6
Earthen jar	32159	10.1	90.3	Tank	1149	33.5	69.1
Bucket	16659	5.2	95.5	Drum	515	14.9	84.0
Drum	9712	3.1	98.6	Earthen jar	126	3.7	87.7
Flower pot	1852	0.6	99.2	Bucket	21	0.6	88.3
Outdoor				**Outdoor**			
Tank	721182	46.1	46.1	Earthen jar	945044	86.2	86.2
Earthen jar	328079	20.9	67.0	Tire	38151	3.5	89.7
Bucket	192673	12.3	79.3	Can & bottle	33059	3.0	92.7
Drum	141372	9.0	88.3	Tank	30380	2.8	95.5
Tire	48211	3.1	91.4	Drum	4464	0.4	95.9
Can & bottle	47239	3.0	94.4	Bucket	1452	0.1	96.0
Rooftop				**Rooftop**			
Bucket	28113	71.1	71.1	Flower pot	2888	72.8	72.8
Tire	3294	8.3	79.4	Tire	465	11.7	84.5
Drum	2996	7.6	87.0	Bucket	420	10.6	95.1
Flower pot	2521	6.4	93.4	Earthen jar	85	2.1	97.2
Earthen jar	841	2.1	95.5	Drum	12	0.3	97.5
Overall				**Overall**			
Tank	976473	50.8	50.8	Earthen jar	945255	85.6	85.6
Earthen jar	361079	18.8	69.6	Tire	38638	3.5	89.1
Bucket	237444	12.3	81.9	Can & bottle	33111	2.9	92.0
Drum	154080	8. 0	89.9	Tank	31562	2.8	94.8
Tire	51696	2.7	92.6	Drum	4991	0.4	95.2
Can & bottle	48217	2.5	95.1	Bucket	1893	0.2	95.4
Flower pot	7788	0.4	95.5	Flower pot	10019	0.9	96.3

Table 3.4: Risk factors for houses of being infested with *Aedes* larvae

	Number of houses	Number of infested houses (%)	Unadjusted OR[†]	95% CI	*P*-value [σ]	Adjusted OR[‡]	95% CI	*P*-value [σ]
Type of houses								
Independent houses	1890	352 (18.6)	**1.56**	**1.35 – 1.82**	**<0.001**	**1.57**	**1.35 – 1.83**	**<0.001**
Semi-permanent houses	2801	364 (13.0)	1.02	0.88 – 1.18	0.78	1.12	0.96 – 1.29	0.16
Slum houses	771	110 (14.3)	1.01	0.57 – 1.79	0.96	0.96	0.54 – 1.70	0.89
Multi-storey houses	3651	466 (12.8)	**1**			**1**		
Had water storage system								
Yes	2575	437 (17.0)	**1.50**	**1.29 – 1.75**	**<0.001**	**1.55**	**1.33 – 1.82**	**<0.001**
No	6647	869 (13.1)	**1**			**1**		
Used mosquito spray/coil/smoke								
Yes	3914	553 (14.1)	1.01	0.89 – 1.13	0.94	–	–	–
No	5281	753 (14.3)	1					
Used insecticide during the last 1 month								
Yes	6747	969 (14.4)	1.06	0.93 – 1.27	0.36	–	–	–
No	2475	337 (13.6)	1					
Had fully/partly-shaded outdoor premises								
Yes	4065	695 (17.1)	**1.55**	**1.38 - 1.75**	**<0.001**	**1.51**	**1.34 – 1.70**	**<0.001**
No	2759	611 (22.1)	**1**			**1**		

[†]Univariate logistic regression analysis

[‡]Multivariate logistic regression analysis adjusted by the variables with $p < 0.1$ (i.e., type of houses, had water storage system, had fully/partly-shaded outdoor premises)

[σ]Wald chi-square test

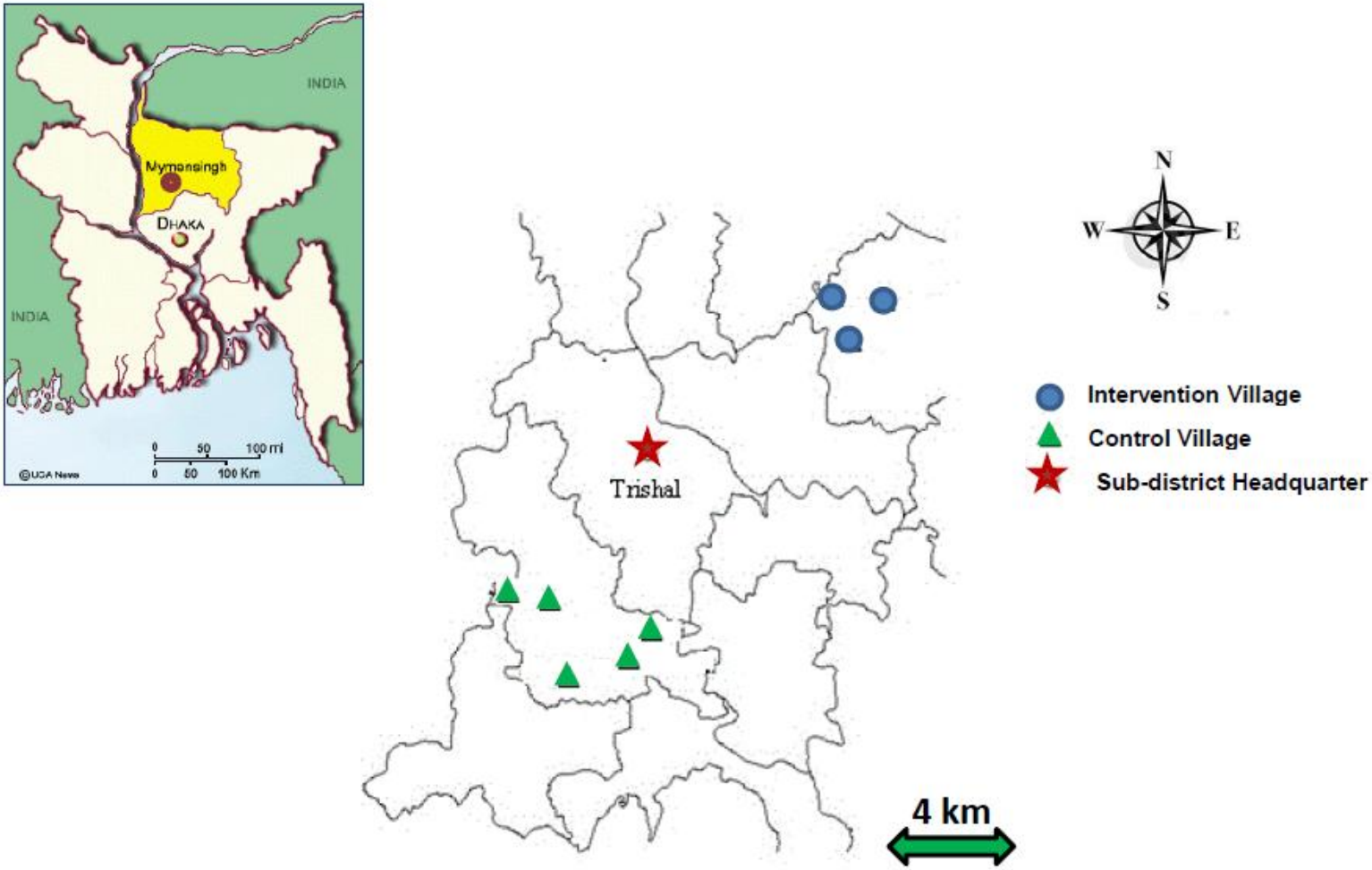

Figure 2.1: Study area

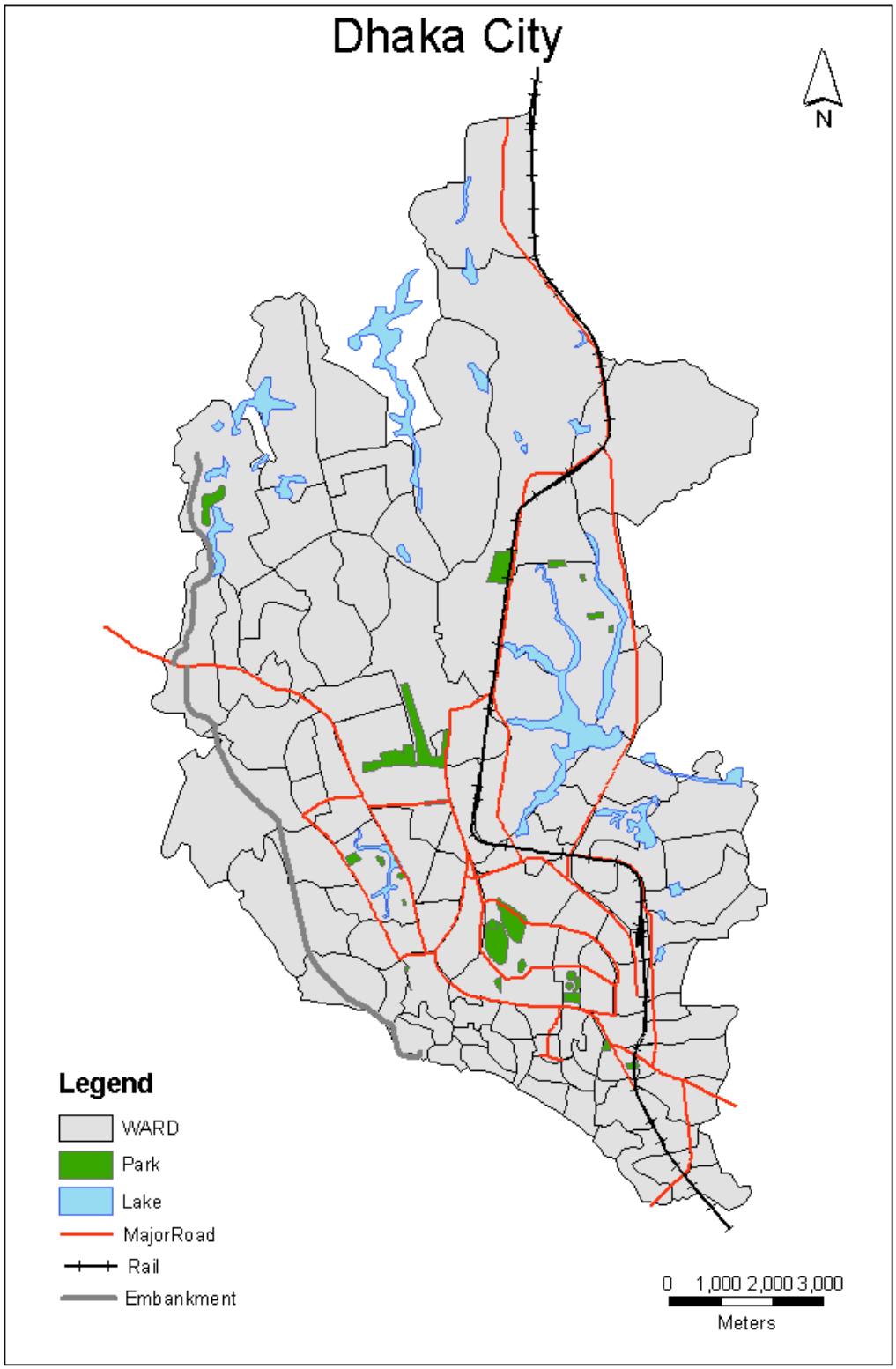

Figure 3.1: Dhaka City

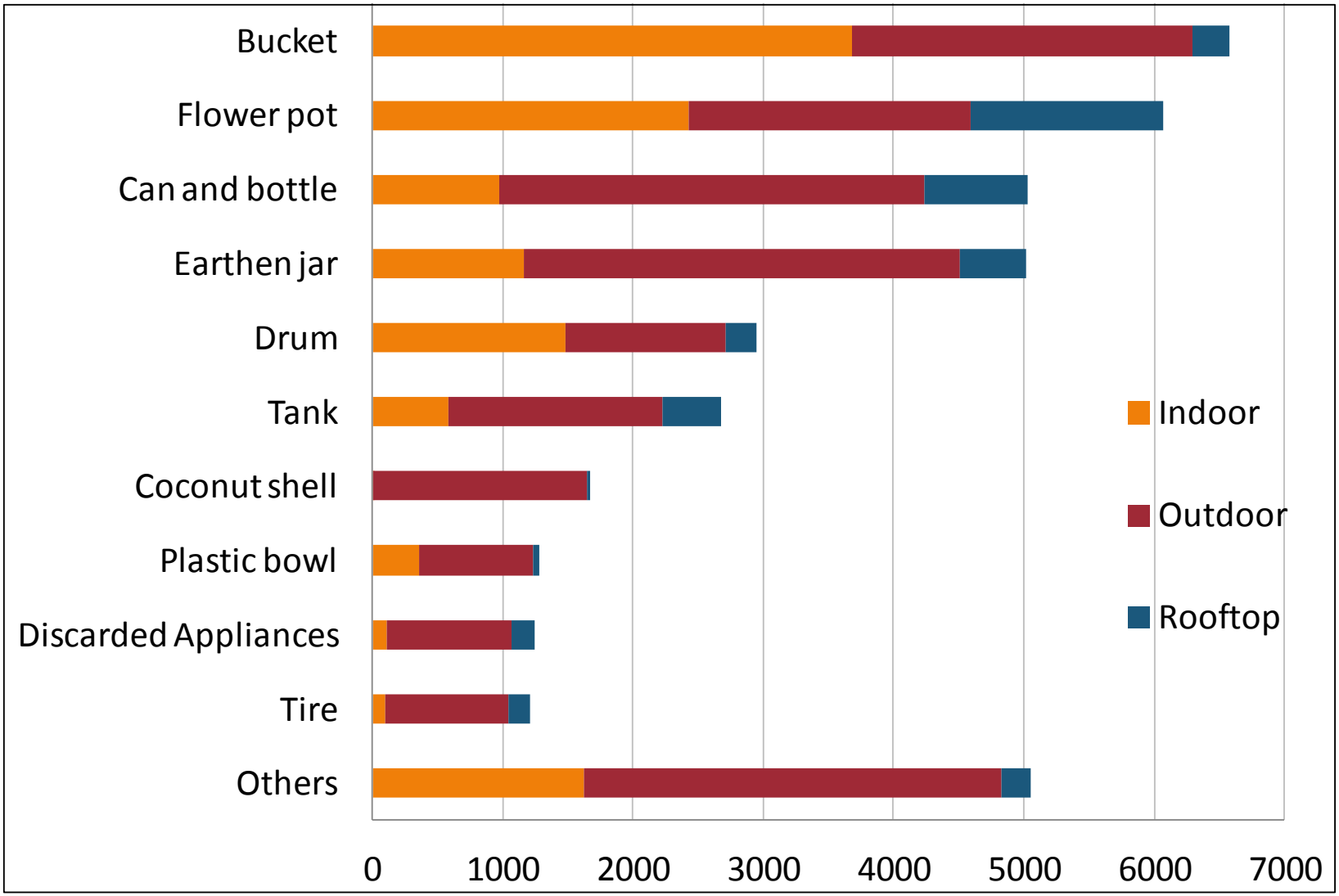

Figure 3.2a: Number of wet containers at different locations

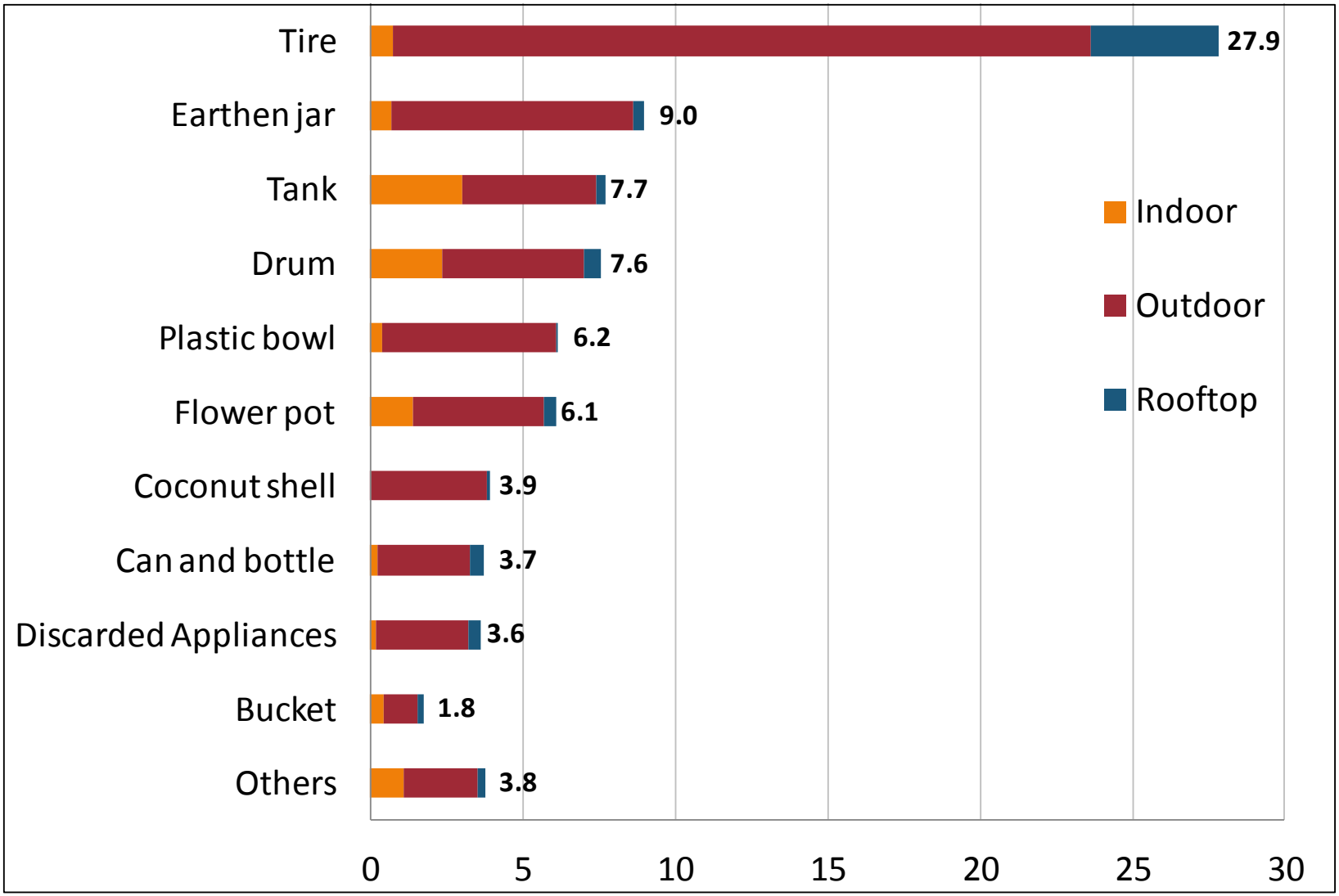

Figure 3.2b: Percentage of wet containers infested with *Aedes* larvae

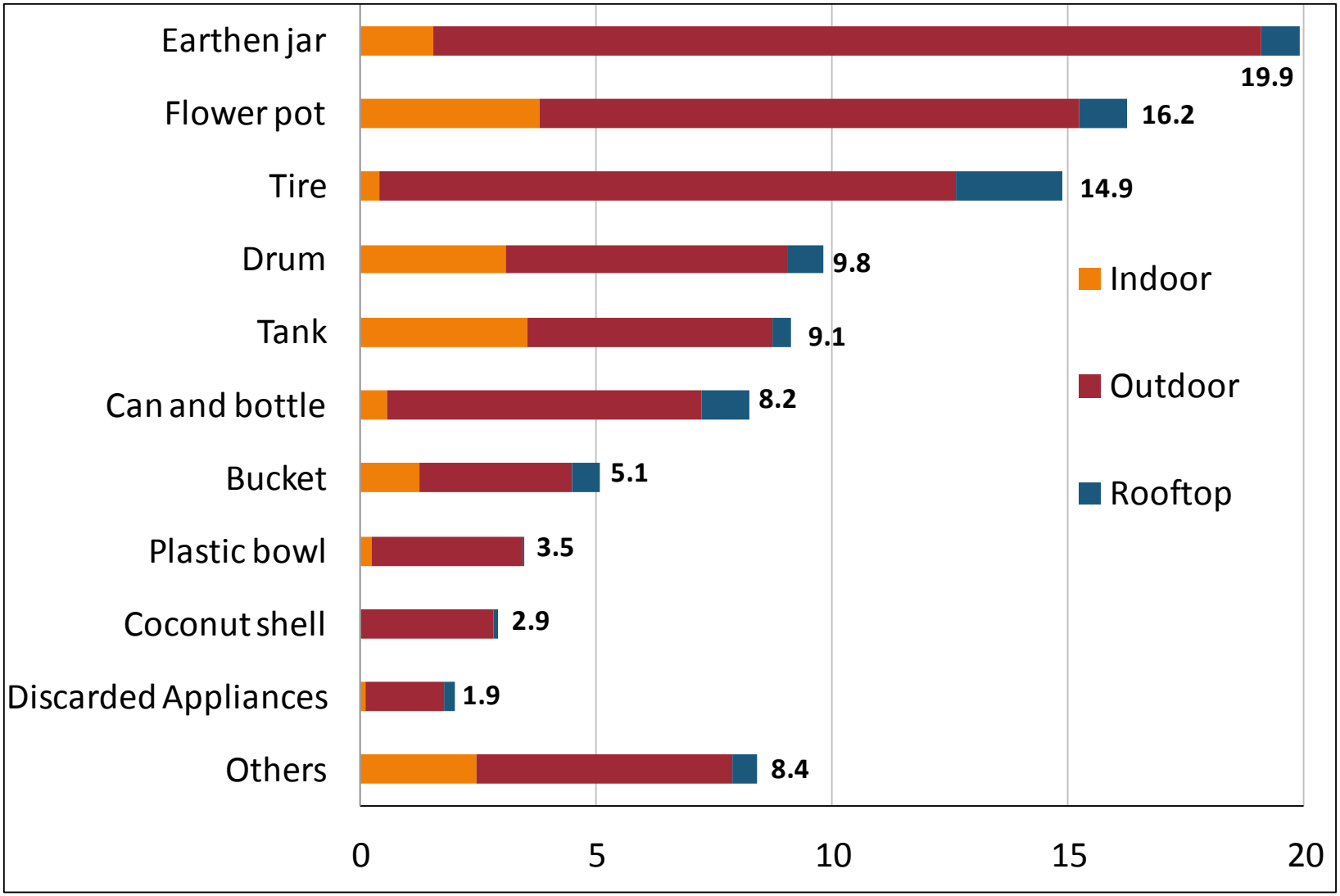

Figure 3.2c: Positive percentage of wet container

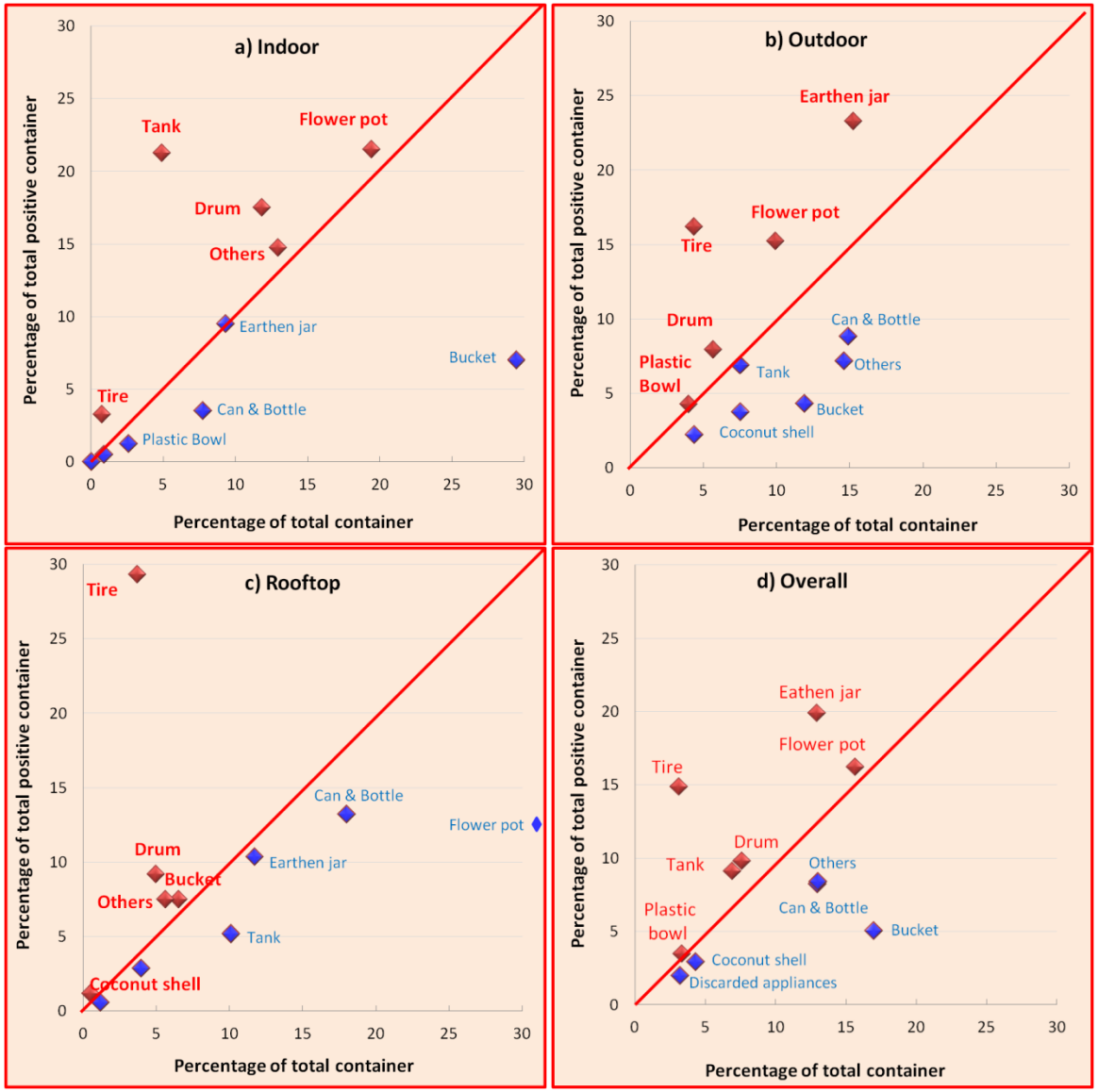

Figure 3.3: Two-dimensional presentation for relative risk of wet containers; a) indoor, b) outdoor, c) rooftop, d) overall

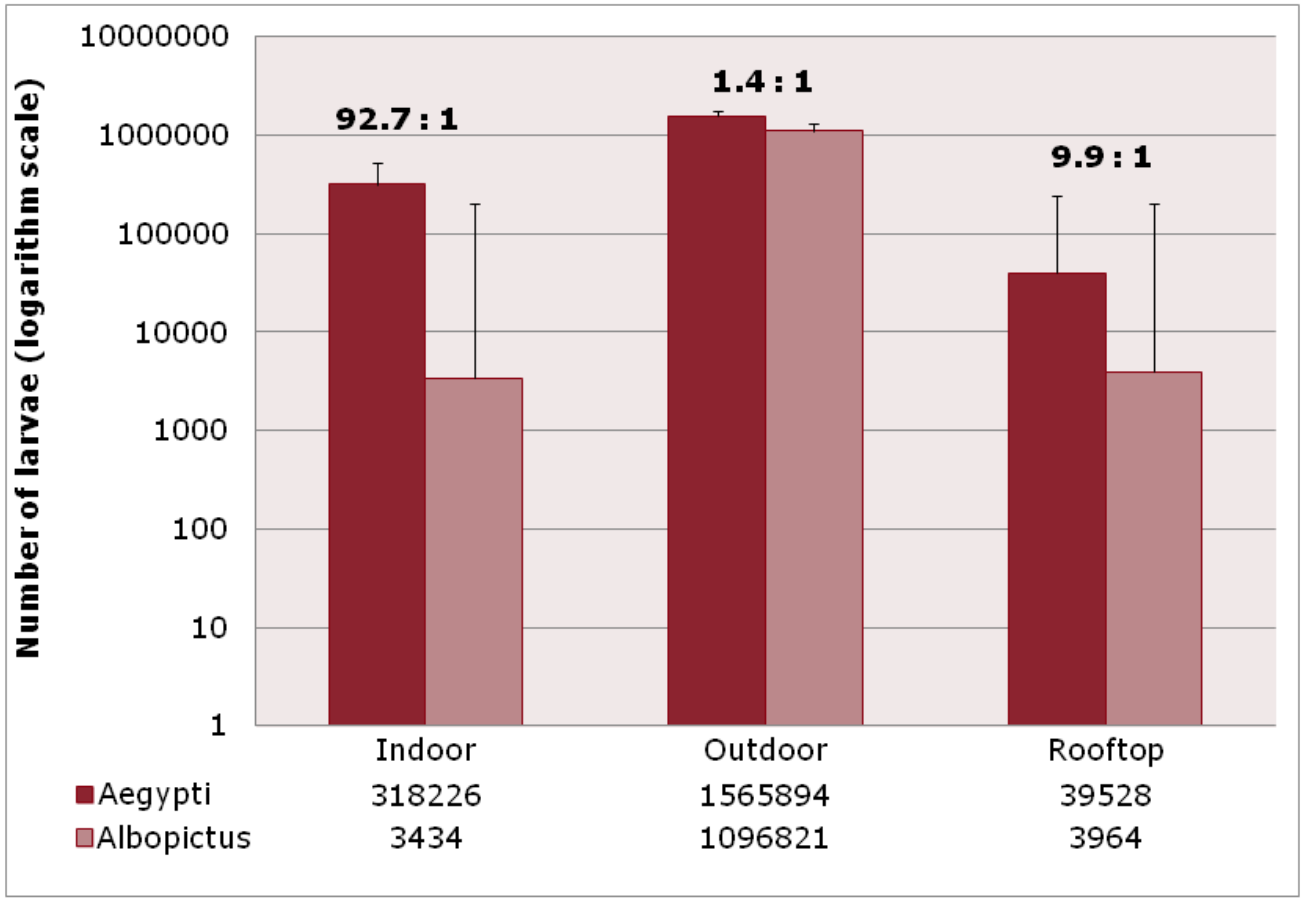

Figure 3.4: Number of *Aedes* larvae by locations

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参　考　論　文