### academic Journals

Vol. 7(33), pp. 4220-4227, 16 August, 2013 DOI: 10.5897/AJMR12.2190 ISSN 1996-0808 ©2013 Academic Journals http://www.academicjournals.org/AJMR

Review

# Challenges and future prospects for Dengue vector control

Iram Liaqat<sup>1</sup>\*, Nusrat Jahan<sup>1</sup>, and Syed I. Ahmad<sup>2</sup>

<sup>1</sup>Department of Zoology, Government College University, Lahore. Pakistan. <sup>2</sup>Institute of Microbiology, UAF, Faisalabad 38040, Pakistan.

Accepted 29 May, 2013

Dengue is a significant public health issue in urban and suburban areas. It is a viral disease transmitted by *Aedes* mosquitoes. Due to lack of specific antiviral treatment or vaccine against dengue, vector control presently appears to be the only effective method for dengue prevention and control. Various methods are in use for vector control depending upon geography and climatic conditions of the endemic areas. Although still widely used are chemical, biological and environmental management techniques, the overall problem remains very challenging. This is due to several limitations associated with existing vector control strategies in terms of cost, delivery and long-term sustainability, however, several new innovative tools are being developed. For example, the release of mosquitoes carrying a dominant lethal system or those harboring *Wolbachia*, that interferes with dengue virus transmission are in the pipeline. Very often vector control programmes involve the use of entomopathogenic bacteria including *Bacillus sphaericus* and *Bacillus thuringiensis* which produce highly potent toxins, targeted specifically against mosquitoes larvae. Any vector control strategy should be selected in accordance to scientific evidence and appropriateness for the epidemiological setting. The current review aimed to discuss the merits of various approaches with reference to vector control required for effective dengue outbreak prevention and control.

Key words: Dengue, control methods, B. thuringinesis, B. sphaericus, Insecticides.

#### INTRODUCTION

In the context of morbidity and mortality, dengue is the most widespread and rapidly expanding vector borne viral disease in tropical and subtropical regions especially Asia, Africa, and the Americas. This arbovirus disease is caused by four related but distinct dengue viruses (DENV 1-4), an RNA flavivirus which has resulted in increased illness and death in Pakistan especially during the last two years. It has been estimated that dengue fever kills approximately 12,500 people every year (Gilbert, 2011). Unlike malaria, bed nets are not effective against dengue as the causative insect vector is active during the day. Excellent adaptation of *Aedes aegypti* to human habitats

is responsible for spread of dengue and dengue haemorrhagic fever (DHF). After infecting host, it remains virulent for the rest of the life. Though the mosquito mainly relies on rainfall for breeding, however it has shown wide adaptation to urban and suburban areas where it uses tyres, rock pools, clay jars, vases, leaf axils, treeholes and even discarded plastic bottles with just a few drops of water remaining. An effective vaccine against the dengue virus is still in development and not yet available; therefore, controlling the *Aedes* vector is currently the only way to reduce viral transmission (Kosiyachinda et al., 2003).

#### EFFECTIVE MEASURES FOR DENGUE CONTROL

Controlling dengue disease can actually be accomplished in three different ways.

1. First available strategy is eliminating the mosquito (*Aedes* spp.) vector by larviciding and adulticiding. Although integrated vector control is appropriate and effective, it may be difficult considering political, geographical and logistical basis, since resurgence of both the mosquito vector and DENV has been observed in areas where these were assumed to be controlled successfully (Sihuincha et al., 2005; Erlanger et al., 2008).

2. Vaccination, a second effective measure to control the disease by administering effective vaccine is currently being tried by both government supported entities and pharmaceutical companies. The development of vaccines against dengue is an important research area however; it is somewhat complicated. This is due to the fact that dengue fever is caused by four different serotypes and there is lack of a suitable animal model for monitoring the progression of dengue disease (Sabchareon et al., 2012). 3. Drug therapy, the third method is based on the use and application of suitable drugs and pharmaceuticals including natural products that may be effective against the dengue fever (Erlanger et al., 2008). Research in this area is currently in progress but no effective remedies have yet been found.

Accordingly, this review will discuss the first and foremost way to control dengue by applying various approaches for vector control.

#### METHODS FOR DENGUE VECTOR CONTROL

A variety of methods have been adopted to control *Aedes* mosquitoes depending upon the severity of the dengue vector in different countries. The important factors which determine control strategies are; (1) ecology of vector, (2) availability and practicability of resources, (3) culture of the affected country. Above all, it is important to determine the ecology of vector to apply the most suitable control method, which may be chemical, environmental or biological in nature. However, depending upon the severity of disease, several complementary intervention strategies including microbial and biological control measures, environmental management and reduction of human-mosquito contact based on community participation may also be applied as integrated vector control (Erlanger et al., 2008; Ahmed et al., 2012).

#### **Chemical control measures**

Most chemical agents including insecticides,oil and sulfur fumigation are used to control predominantly the adult stage mosquitoes or vector breeding sites in several countries of the world. Several products including smethoprene and pyriproxyfen, the chitin synthesis inhibitors triflumuron and novaluron, and the organophosphate temephos and synthetic pyrethroid insecticides offer residual control of A. aegypti and reduce overall populations seasonally and for outbreak control (Scott et al., 2010). In Cambodia, for example, larvicides are being used before start of dengue season in densely populated areas as a short-term intervention since the year 2000. As a result, dengue cases and deaths were reduced by 53% (Suaya et al., 2007). To prevent outbreak, this exercise is repeated twice annually. However, in larger countries, it is not possible to target every possible breeding container due to cost and operational delivery shortfalls. Hence, larvicides should be used in tandem with community mobilization preventing environmental damage. While larvicides are effective in lowering vector density, infectious adult mosquitoes are not affected, eventually developing resistance against these chemicals and transmission may continue for the remaining lifetime of the infectious adult mosquito (Scott et al., 2010). Moreover, they also have certain unwanted effects, such as their non-selectivity which very often causes environ-mental damage. With increase in public awareness about environmental issues, the rules for application of chemicals have been tightened and effectively monitored (Kosiyachinda et al., 2003).

#### **Environmental control measures**

Environmental control measures aim to change the environment in such a way that vector breeding sites, especially in close proximity to humans should be lessened. thus leading to minimize human-vector contact. These modifications may be categorized into long-lasting and short-term measures. Long lasting measures include modifying building designs, preventing female Aedes entry and repairing blocked cement drains. Short-term environmental modifications involve engagement of the community and local agencies. Households often feature water storage containers placed outside. These containers store rain, river or well water for household use and drinking water for animals and are extremely difficult to protect from A. aegypti infestation. In addition, discarded containers, tyres and other vessels collect rainwater during the rainy season, providing excellent sites for A. aegypti breeding. Household sanitation is important to control Aedes breeding in domestic environments. Other potential breeding sites are containers found in parks, empty land, industry buildings, construction sites, blocked cement drains and septic tanks. So the local authorities should make sure that there is no accumulation of water containers in public places (Eisen and Lozano-Fuentes, 2009).

#### **Biological control measures**

Biological control is one of the alternatives for overcoming the problem of insecticidal resistance resulting from the indiscriminate use of chemical pesticides and their harmful consequences for the environment both in agriculture and in public health. Biological control approaches are preferable due to advantages they offer, particularly specificity and safety for man (Lacey, 2007). Predatory fish (Ghosh et al., 2011), application of copepods (Horstick et al., 2010), plankton management and Bacillus spp. (Ritchie, 2005; Ritchie et al., 2010), have been tested as biocontrol agents in immature stages of the Aedes mosquitoes. Most control agents are comparatively safe, effective, economically produced and are relatively easy to administer on a large scale and acceptable to the target population both culturally and socially (Lacey, 2007). Stagnant water reservoirs in the rural areas and the open waste water drains/canals in the urban areas are difficult to eliminate therefore, promoting potential threats of mosquito born infections like dengue and malaria in the community of population. A combination of B. thuringiensis and *B. sphaericus* spore and crude toxins suspension in the form of Biomoskill-Plus (BMK-P) impre-gnated bricks may offer a suitable mosquito-lavicidal activity in such environment without harm to the animals and birds or fish population with Prof. Sajjad-ur-Rehman at University of Agriculture, Faisalabad. A project in this regard was completed successfully by him and process of comercialization of bricks is in progress.

#### Larvivorous fish and predatory copepods

In the case of *Aedes* mosquitoes, certain species of fish and predatory copepods have been reported to be very effective. *Poecilia* and *Gambusia* are the most preferred poeciliid larvivorous fish tested against *A. aegypti* larvae. *Poecilia* is an omnivorous species that survives well in confined habitats, for example open dug wells. In case of the absence of available larvae, this fish spp. can survive on other available food sources also. It grows equally well in small containers. In contrast, *Gambusia* is a canniballistic fish species that feeds on zooplankton preferably, and its populations cannot survive in smaller water bodies for long duration (Ghosh et al., 2011).

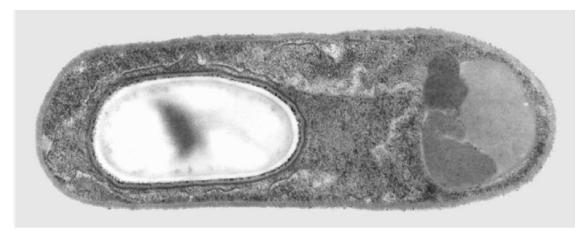
Copepods are small aquatic crustaceans which prey on first-instar mosquito larvae making them potential biological control agents recognized as early as in 1981 (Riviere and Thirel, 1981). Since then, various species of copepods have been tested for their effectiveness to control mosquito larvae. Many of these are omnivorous and prey on especially first-instar larvae, but very few on later stages. Various species of copepods, including M. thermocyclopoides, M. longisetus, M. guangxiensis, Mesocyclops aspericornis and M. aspericornis, have been observed as effective biological control agents of A. aegypti (Kosiyachinda et al., 2003). In Vietnam, populations of local Mesocyclops spp. were included in specifically designed community-based control programmes supported by Micronecta water bugs and fish to control the dengue problem (Nam et al., 2000). Wang et al.

(2000) carried out studies on integrated control of Aedes utilizing G. affinis, P. reticulata, Tilapia mossambica and Sarotherodon niloticus in potable water containers in a coastal village in Taiwan. These fish spp. were later replaced with Cyprinus carassius because of their easy availability and adaptability to local environmental conditions. In 1980s, Chinese catfish was also used to control A. aegypti larval breeding to overcome dengue outbreak which occurred in fishing villages in Chinese coastal provinces (Wu et al., 1987). In Thailand, the most successful way to exercise control over A. aegypti was by using fish in rectangular tanks and by properly covering water-storing containers with suitable lids (Phuanukoonnon et al., 2005). MartiNez-Ibarra et al. (2002) reported five indigenous fish species, namely Lepisosteus tropicus, Astyanax fasciatus, Brycon guatemalensis, Ictalurus meridionalis and P. reticulate to be effective against A. aegypti larvae in water storage tanks in Southern Mexico. Pamplona (2006) used five non-native larvivorous fish species (Betta splendens, Trichogaster trichopteros, Astyanax fasciatus, P. sphenops, and P. reticulata) to combat A. aegypti larval infestation in the northeastern Brazilian state of Ceará. Likewise, Seng et al. (2008) reported another successful example in Cambodian villages, where 79% decrease in dengue carrying A. aegypti larval infestation was observed compared to control villages.

Specifically targeted predators present potential for consideration in the biological control of mosquitoes. However, owing to their stringent ecological requirements, predators can only survive provided their preferred living conditions are actively met, a condition not easily achievable. Furthermore, the life cycle of the predator needs to be well adopted or suitable to dove-tail with the requirement of the prey. Another challenge is the mass rearing and release of the predators or parasites, which is usually expensive or mostly not easy to achieve. Accordingly, large-scale use in specific habitats is often restricted and hence special attention must be given to the search for microbial control agents, particularly bacteria of the genus *Bacillus*.

## Entomopathogenic bacilli as potent vector control agents

At present, only two types of entomopathogenic bacilli are being used in control programs in the field, *Bacillus thuringiensis var israelensis* and *Bacillus sphaericus*. Both are considered as the most potent and successful groups of organisms to be utilized. Their specific properties including (i) high efficacy against target organisms, (ii) relative ease for mass production on industrial scale, (iii) environmental safety, (iv) cost- effectiveness, (v) minimal development of resistance, and (vi) simple integration into control programs involving community participation make them potentially the most effective biocontrol agents. The insecticidal activity is based on the occurrence of proteinaceous parasporal crystalline inclusions



**Figure 1.** *B. thuringiensis israelensis* with spore (left) and parasporal inclusion of crystal structure (Micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris).

(the so-called protein crystals) composed of potent mosquito-toxic proteins (Gammon et al., 2006). The above mentioned two *Bacilli* species differ from each other in their properties as further discussed.

#### Bacillus thuringiensis israelensis (Bti)

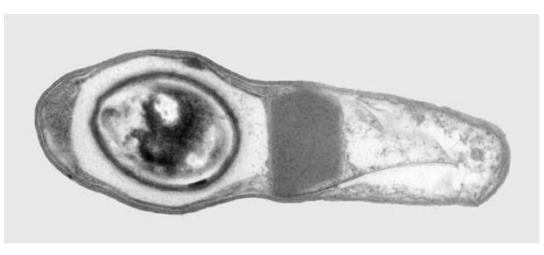
Goldberg and Margalit (1977) isolated a strain in Israel from moribund larvae of Culex pipiens that showed toxic activity against Diptera. It was identified and named as B. thuringiensis var. israelensis (Bti), which became the first bacterium used in biological control programs against Diptera. In Pasteur Institute, France, this strain was positively identified as B. thuringiensis serotype H-14. It exhibits high toxicity to mosquito larvae of the genus Aedes and Culex, while it is less active against Anopheles. It can be easily isolated from soil, mud, surface of leaves, and warehouses (Hongyu et al., 2000). Insecticidal activity of Bti is related to the production of protein toxins, concentrated in a parasporal inclusion of crystal structure called sigma-endotoxin (Figure 1), which is synthesized during sporulation and is located associated with the spore. Due to the presence of these proteins, this bacterium is highly toxic to mosquito and insect larvae.

*B. thuringiensis var. israelensis* is toxic due to the presence of a 128-kb plasmid, *pBtoxis*, that carries four main insecticidal toxins specific to dipterans (cry4Aa, cry4Ba, cry10Aa, and cry11Aa) and one cytolytic Cyt toxin (cyt1Aa) (Crickmore et al., 1998). The Cry toxins are crystalline proteins that kill insect by binding to specific glycoprotein receptors on the larval midgut brush border and disrupting membrane integrity (Bravo et al., 2007). Cytolytic toxins act synergistically with the Cry toxins, apparently as an alternative non-specific membrane receptor (Manceva et al., 2005). This synergism in the mode of action among proteins is one of the main advantages of this insecticide which reduces the possibility of resistance. However, *Bti* is more sensitive to UV radiation than *B. sphaericus*, and the duration of effective control is shorter, especially in polluted waters (Silapanuntakul et al., 1983).

#### Bacillus sphaericus

*B. sphaericus* has become increasingly important in recent years. It is a potential bacterial control agent due to its spectrum of efficacy (particularly against *Anopheles* and *Culex* species) and its ability to recycle or to persist in nature under polluted water conditions. It also exhibits less UV sensitivity and is toxic largely by virtue of a chromosomally encoded binary gut toxin (Bin) consisting of two proteins of molecular weights, 51.4 kDa and 41.9 kDa, respectively. However, *B. sphaericus* is not effective against one major vector mosquito, *A. aegypti*, and resistance has been found in some mosquito species (Gammon et al., 2006). This bacterium can be easily identified by its round spore located terminally in a swollen sporangium (Figure 2).

It was noted that Bin toxin of *B. sphaericus* is highly synergistic with the Cyt1Aa toxin from *B. thuringiensis* subsp. israelensis (Wirth et al., 2000, 2001). Therefore, resistance to B. sphaericus can be overcome by transferring cyt1A gene from B. thuringiensis strain (Thiéry et al., 1998). Targeted strains can be significantly improved by combining their toxins along with utilizing better environmental conditions. Many researchers have reported enhancing toxicity success of *B. sphaericus* by moving *B.* thuringiensis var. israelensis toxin genes into it (Poncet et al., 1994, 1997). However, only a slight delay in the development of insect resistance was observed by transferring single gene, since it was reported by Georghiou and Wirth (1997) that both Cry and Cyt toxins are necessary to prevent resistance to B. thuringiensis var. israelensis (Bti) toxins. Furthermore, "two helper proteins", P19 and P20, which may stabilize the toxins or aid in crystal formation and presence of all of Bti toxins enhance its optimum toxicity (Wu et al., 1987). Interestingly,



**Figure 2.** *B. sphaericus* with round spore and the parasporal protein inclusion (dark structure on the right site of the spore) which is located in a coated "spore crystal complex" (Micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris).

all the Bti toxins and the two helper proteins are present on a single 128-kb plasmid (Berry et al., 2002). Mosquitotoxic properties of B. sphaericus might be improved by transferring this plasmid, resulting in best properties of the two species in a single organism. Classical microbiological mating method can be used to transfer pBtoxis producing transconjugants. Filter mating methods were employed successfully for conjugal transfer of pAMB1 both within and between B. sphaericus species (Correa and Yousten, 1997). Likewise, conjugation methods using liquid media for both B. thuringiensis var. israelensis (Hu et al., 2005) and B. sphaericus (Correa and Yousten, 1997) in liquid media have also been reported (Wilcks et al., 1998). In the case of B. thuringiensis, in some subspecies the toxin-encoding plasmids themselves are able to conjugate between B. thuringiensis strains (Vilas-Bôasetal., 1998). However, Monneratetal. (2004) observed that some *B. sphaericus* isolates produce protein toxins referred to as the mosquitocidal toxins (MTX) having molecular weight of approximately 100 kDA and are found to be homologous neither to the binary toxin nor to Bti toxins.

It should be kept in mind that while using bacterial control agents, it is necessary to apply effective formulations to target organisms without harming the environment. Various Bti preparations can be obtained as water dispersable granules (WDGs), wettable powders, fluid concentrates, corn cob, sand and ice granules, pellets, tablets or briquets (Becker et al., 2003). Usually a very small amount for example, a few hundred grams of powder, or a few kilograms of granules per hectare, are sufficient to kill mosquito larvae. However, long-term effectiveness can be achieved using larger amounts in the form of tablet or briquet formulations. The tablet formulations, based on Bti or *B. sphaericus* material sterilized by gamma-radiation to prevent contamination of drinking water with spores, is successfully used for control

of container breeding mosquitoes such as *A. aegypti* (Mahilum et al., 2005).

#### Other Mosquitocidal bacteria

Application of *B. thuringiensis* and *B. sphaericus* strains to control successfully mosquitoes and blackfly problems has led to the search for more effective isolates of a variety of other bacteria. For example B. thuringiensis sub sp. Jegathesan discovered in Malaysia, produce mosquitocidal proteins identified to be used as new larvicide (Delécluse et al., 2000). Its larvicidal activity is due to a complex of seven Cry and Cyt proteins, having different toxicological properties, several of which are related to those of Bti. Cry11B, an 80 kDa protein is an interesting example that is approximately 10-fold more toxic to mosquitoes compared to the related Crv11A protein found in Bti (Delécluse et al., 2000). Likewise, Clostridium bifermentans, have been reported to have mosquitocidal properties, identical to those previously reported (Delécluse et al., 2000). Among these, PG-14 isolate of B. thuringiensis subsp. *morrisoni* is an interesting isolate discovered in the Philippines. It exhibits toxicity comparable to Bti and also produces the same complement of endotoxin proteins (Cyt1A, Cry4A, Cry4B and Cry11A) plus an additional 144-kDa Cry1 protein toxic to lepidopterans. All of this information emphasizes that the search for new insecticidal isolates must continue in case if the new strains prove not to be as effective as Bti and B. sphaericus.

#### Initial recombinant mosquitocidal bacteria

Highly mosquitocidal strains of Bti and *B. sphaericus* along with other mosquitocidal bacteria with unique set of toxins has suggested construction of improved recombi-

nant bacteria that best express mosquitocidal properties. This is because, resistance is difficult to develop against multiple toxins with different modes of action compared to a single toxin with the same mode of action. Bti toxins with multiplicity in their structures and different modes of action make them more effective as mosquitocidal bacteria versus B. sphaericus toxin with single spectrum and simple mode of action. Thus, improved recombinant mosquitocidal bacteria can be produced in either of the following two ways: (i) introducing B. sphaericus toxin genes into Bti and (ii) introducing Bti or related mosquitocidal toxin genes into the best B. sphaericus strains. Either of the above strategies can be effective and have been used to produce a variety of Bti and B. sphaericus recombinant strains each exhibiting different protein components.

#### LIMITATIONS OF VECTOR CONTROL MEASURES

Integrated vector control, either by chemical, environmental or biological means, has led many dengue programmes in the past. Despite the fact that it is still widely used and promoted with great success, clean-up campaigns are limited by the level of compliance by the community. Also, the most productive containers cannot be disposed of or emptied (example drinking-water stores). However, such water can be subjected to larvicidal treatment. Only temephos, permethrin, Bti and pyriproxyfen are approved by World health organization for use in drinking-water. Additives with persistent flavour are placed into drinking water which is viewed with suspicion and not well accepted. Furthermore, water usage or emptying of containers on regular basis can lessen the intended effect. In order to improve situation, it is better to focus only on productive sites with application of effective larvicides only. Additionally, targeting of epidemiologically important breeding sites/containers should enable effective control (Nathan et al., 2006).

#### FUTURE PROGNOSTICATIONS AND PERSPECTIVES

Though awareness for dengue prevention is universal, various challenges still remain and need to be addressed. Inadequate funding, limited resources and the lack of a solid strategy to respond to the increasing problem of dengue outbreaks in a growing number of geographical areas are some of the reported problems for dengue control. Apart from those mentioned above, others include basic sanitation problems, rapid urbanization, lack of control due to increased mobility of populations and international travel that has exacerbated the problem in some countries and areas, and therefore, sustainable control of dengue vectors is an arduous task. Hence, there is a need for a well-organized dengue control strategy which warrants interaction and cooperation among different sectors, ministries and agencies to plan and facilitate these activities for successful implementation and outcome.

In case of an outbreak, a response strategy should be pre planned by considering three components at various levels. First, one should consider employing environmental management and other technologies as applicable for community-based larvae control. Second, one should focus on control of adult mosquito population management using novel insecticides and last but not the least is use of repellents alongwith adult population reduction devices for personal protection.

Preventive activities should be built into existing health care systems and should be well coordinated within primary health care activities. Integrated Vector Management (IVM), a further method of dengue vector control has been recommended by World Health Organization which included legislation, community mobilization, collaboration within the health delivery system as well as other sectors. It is ideal to manage and control dengue in dengue endemic countries and should be used for planning and implementation to prevent dengue in every region of the country (Ahmed et al., 2012). New vector control tools for A. aegypti population suppression and replacement are progressing. Sterile Insect Technique has been used for insect population control against a wide range of agricultural pest insect species (Krafsur, 1998). Another new technology involves Release of Insects carrying a Dominant Lethal (RIDL) system for mass rearing of male mosquitoes (Alphey et al., 2008). Using this advanced method, a lethal gene was micro-injected into the eggs of A. aegypti. Subsequent integration of such a lethal gene into the genome of mosquito regulates the production of toxic metabolites in the larval stages, resulting in dead larvae. Such mosquitoes are reared and maintained in the laboratory using tetracycline. This antibiotic is necessary for inhibition of lethal gene and allows larvae to develop into adults. In field trials, RIDL males are released to mate with wild females. As a result, females produce eggs with RIDL gene, hence it results in death at late larval or early pupal stage.

Another important discovery involving novel vector control is the use of endosymbiotic bacterium Wolbachia, usually found in insect populations. It can interfere with replication of the dengue virus in *A. aegypti* mosquitoes (Jeffery et al., 2009). A study by Yeap et al. (2011) reported Wolbachia popcorn (wMelPop) as an agent for preventing dengue transmission by reducing life span and interfering with viral transmission. Using another strain of Wolbachia species (Wolbachia pipentis), a recent study published it as an effective measure against Aedes since it does not affect the viability of vector and has high maternal transmission thus providing ideal condition for invasion (Walker et al., 2011). However, it is worth mentioning here that Wolbachia doesn't eliminate the replication of dengue virus. Instead it limits viral replication. There is a distinct possibility that in future this virus will develop advanced replication phenomenon making it more virulent to survive under selection pressure exerted by Wolbachia. So there still remains the need for developdeveloping new and better strategies to control the vector until a suitable vaccine is developed and marketed.

There is also a further need to improve vector surveillance by regulating data on key container types for better management of breeding sites. In order to identify key patterns and relationships relevant spatial data and geographic information system (GIS)-based approach must be available and utilized which will aid in planning and strategic decision-making processes (Eisen and Lozano-Fuentes, 2009). Thematic maps to visualize the spatial patterns of dengue distributions over time and to monitor potential movements of dengue transmission foci after outbreaks can be produced in relation to relevant environmental and climatic indicators. Recent data have clearly shown that global warming and climate change scenarios have also fueled a global rise in dengue cases reported annually to WHO between 1955- 2005 (Kroeger and Nathan, 2006). During this period, dengue cases increased by 1.000 fold (Hales et al., 2002). Accordingly, we will need all our resources and variety of new approaches as outlined above to equip our programme managers with new and powerful knowledge based decisionmaking, planning, surveillance and community education tools to succeed in our quest for effective dengue control.

#### CONCLUSIONS

Although intense efforts have been made to develop a vaccine, there is still neither an effective vaccine nor an anti-viral drug at hand to treat the disease. It is likely that a promising vaccine may take several years to reach the larger populations in developing countries. Hence, to minimize the impact of dengue outbreaks, countries and regions need to develop a suite of strategies for long term vector control. Integrated approaches with evidence–based selection and delivery of different intervention programmes to different entomological and epidemiological settings including a variety of control methods will be required. Dengue prevention and control can be achieved effectively by the active and resolved participation of individuals, families and community with the help of local, state officials and allied health personnel.

#### ACKNOWLEDGEMENT

This project was funded by EFS-USDA, University of Agriculture, Faisalabad.

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