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USE OF GENETICALLY MODIFIED MOSQUITOES TO FIGHT DENGUE IN BRAZIL

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ABSTRACT

Mosquito-borne diseases are one of the major barriers preventing economic progress in the developing world. According to the World Health Organization, 200 million people were victims of malaria in 2010 and 655,000, mostly children, died from it. Dengue fever is believed to affect 50-100 million people per year and results in around 20,000 deaths. Dengue is the most important mosquito-borne, human viral disease in many tropical and sub-tropical areas. In Brazil the disease has been essentially described in the form of case series. Despite the presence of dengue in Brazil since the early 1981s, dengue has become a major public health issue, with a high morbidity and mortality. Aedes aegypti and Aedes albopictus are the vectors responsible for the transmission of dengue viruses (DENV). The genetically modified (GM) mosquitoes being used in these field experiments and cited alternative methods for dengue control. This technology and its impact to the environment studies have focused on controlling the mosquito populations by genetically modifying the insects. Tactics to protect people in endemic areas such as stopping mosquito bites using insecticides, net and repellents, developing preventive drugs and health education to manage mosquito-borne diseases have not shown full effectiveness.

KEYWORDS

Dengue fever, Aedes aegypti, Genetically modified mosquitoes and Brazil.

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INTRODUCTION

Dengue virus is one of the viral diseases transmitted by the mosquito vector *Aedes aegypti*, it is an arbovirus. It is a related yellow fever disease that is also transmitted by the same mosquito. The virus infects only certain hosts as men and monkeys[1]. It produces a rank of several clinical symptoms from a similar syndrome to a grave flu or sometimes a dengue hemorrhagic fever (DHF)[2]. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people, over 40% of the world's population, are now at risk from dengue. WHO currently estimates there may be 50–

100 million dengue infections worldwide every year[3]. Dengue fever is transmitted to humans through the bites of female Aedes mosquitoes. When a patient suffering from dengue fever is bitten by a vector mosquito, the mosquito is infected and it may spread the disease by biting other people. The disease cannot be spread directly from human to human. In Hong Kong, the principal vector *Aedes aegypti* is not found, but the prevailing species Aedes albopictus can also spread the disease[4]. There are four antigenically related, but distinct, dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), all of which can cause DF/DHF. Recovery from infection by one provides lifelong immunity against that particular serotype. However, crossimmunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing sever dengue[3]. Previously DEN 2 serotypes were more common but recent studies have shown increased prevalence of serotypes DEN 1 and DEN 3[5,6]. In Brazil DEN 4 serotypes have been more prevalent today[7]. Dengue virus infections may be asymptomatic or lead to a range of clinical manifestations, even death. The incubation period is 4-7 days (range 3-14). Typically DF is an acute febrile illness characterized by frontal headache, retroocular pain, muscle and joint pain, nausea, vomiting and rash[8,9]. The febrile, painful period of DF lasts 4–7 days, and may leave the patient tired for several more days. A biphasic or "saddleback" fever curve is not the norm. Dengue virus disappears from the blood after an average of 5 days, close correlated with the disappearance of fever, and no carrier states ensues[10,11]. The WHO and US Centers for disease control have considered dengue fever a major health threat for Brazil, Pakistan and India[12]. The absences of specific vaccine and anti-viral treatment of DF are the main reason for making it a global problem[13,14]. Several prevention policies are employed along with health education, millions are spent on television media to try to educate the public that whenever the rainy season in which the disease intensifies over, most people are not aware and still leaves tires, containers and etc., favoring the accumulation of stagnant water, stimulating the development of the larva. To the Minister of Health, Alexandre Padilha, the strategy to fight dengue by controlling mosquito is outdated. He said the new focus of the folder is on the integration of health care actions such as reducing the waiting time for diagnosis and initiation of treatment.

Dengue in Brazil

Dengue virus belongs to family Flaviviridae, having four serotypes that spread by the bite of infected Aedes mosquitoes.86% of the cases registered in 2007 occurred in the rainy season between January and May when temperatures are on average over 20^{o}C and humidity is high, the perfect conditions for dengue transmission. Brazil has a population of 192.9 million, with a large proportion of this number living in conurbations such as Rio de Janeiro and Sao Paulo. Most of Brazil's cities have an endemic population of *Aedes aegypti* as they are often densely populated with open air water storage facilities providing ideal breeding sites for the dengue mosquito. In these cities, the incidence rate of dengue fever can be as high as 268.2 per 100,000 inhabitants compared to the national average of 34.5 cases per 100,000 inhabitants. The first recorded outbreak of dengue fever in Brazil occurred in 1981 in Roraima. Brazil is a hotspot of dengue fever, which is one of the most serious endemic diseases in terms of public health and economic costs. This disease has burden and attained an unprecedented proportion in recent times with sharp increase in the size of human population at risk. It presents highly complex pathophysiological, economic and ecologic problems. In Brazil, the introduction of DENV-1 and DENV-2 in 1986 and 1990, respectively, both through the State of Rio de Janeiro resulted in several epidemics occurring almost all over the country. In the following eighteen years it has become hyperendemic for dengue fever with all four serotypes circulating in the country. Periodic epidemics of the different serotypes have occurred in 1987 and 1991. The dengue pandemic occurred in 1998, Brazil has experienced with a peak in the number of cases and an expansion in the geographic range of the virus. By 2000 all 26 states had reported cases of dengue fever with five million cases reported from 1985 to 2008. The number of cases each year varied between 113,000 and 781,000 during the period of 1999 to 2009 with a general upwards trend. The rate of secondary infection by DEN-2 and DEN-3 in Brazil is extremely high leading to an increasing number of DHF/DSS cases. Until its reintroduction in 1976 the *Aedes aegypti* population had been largely controlled by the efforts of the Pan-American Health Organization (PAHO) to eliminate yellow fever using DDT and other insecticides. However, from 1986 a lack of funds forced a policy change from focusing on eradication to control of the mosquito population, and the entomological surveillance

systems were allowed to decline. Subsequently the dengue vector infestation rapidly expanded demonstrating the failure of the control methods. In 1996 a new plan for the eradication of the mosquito was initiated incorporating chemical insecticides, sanitation and education. Rather than a top down government project the eradication programme therefore focused on social mobilization as a vehicle to reach its aims. However, administrative difficulties prevented the programme from reaching its expectations. Instead, from 1997 to 2001 a project based solely on chemical vector control was implemented. During the last years a large number of physicians have treated and described dengue disease in Brazil, but the scientific studies addressing various problems of dengue disease have been carried out at limited number of centers. Scientists have added a gene to the dengue-spreading insect which renders it capable of destroying its own species. Bio control is another method for controlling mosquitos. Natural predators are introduced to prey on mosquitos. Dragonfly naiads and the mosquito fish eat mosquito larvae. However, the predators may not be suited to the environment. Mass-breeding and release of the predators is often expensive or impossible. Although ecologically friendly, for these reasons bio control is not a viable scheme for large-scale mosquito control. The mosquito control has approached vigilance and control programs of *Aedes aegypti*, which are included the environment cleaning systems, with a very active participation of the community, in order to reduce them of the breeding places. However, these measures have not been enough for the larva population control, in the last 15 to 20 years; organophosphate insecticides have been used, included grainy temephus for its use domestic waters containers for the larva state and Malathion and fention in the adult state. Nevertheless, the problem of the use of insecticides is the resistance of almost all the insecticides[15]. Many years ago the Luke Alpey group of the University of Oxford in England, have been working in the idea of insect's genetic control and recently they found associated with its collaborators the Oxitec Company, which produce a strain OX513A of transgenic mosquitoes RICDL of *A. aegypti* which takes a dominant lethal gene which acts to larva level. The mosquito can be reproduced in the laboratory because the expression of the lethal gene is "turned off" in the presence of an antibiotic (tetracycline), however, once released he homozygous transgenic mosquitoes which are crossed with wild females, the entire descendants die in the larva state because of the tetracycline is not present [16].These tests have been taken to the field, specifically in the Cayman Islands; where around 1 million of mosquitoes were released and as a result of that the population as reduced until 10% of the initial [17]. In Brazil the technology has been tested in neighborhoods of Mandacaru and Itaberaba in the town of Juazeiro, Bahia, in 2011 and 2012. More ambitious collaboration with Oxitec is in the "ramp-up" stage of building a "mosquito plant" in the state of Bahia. Eventually, 4 million altered male mosquitoes will be released. The results showed a decrease of about 90% in the incidence of mosquito that was very high in both locations. In July 2012, Health Minister Alexandre Padilha opened a plant of transgenic sterile males in the city with the capacity to produce four million of them per week. The first place to get them will be Jacobina in Bahia, a municipality with 79,000 inhabitants which had 1,647 dengue cases and two deaths due to it only in the first half of 2012 [18].

The GM mosquitoes are all male. The insects have been engineered to be infertile in a method known as sterile insect technique (SIT) (Figure No.1). Released into the environment, they mate with the native females and pass on their genetic fault to the offspring. The female lays eggs that develop into pupae but the faulty gene causes them to die before reaching adult stage. Female mosquitoes only mate once in their lifetime. If they do so with the sterile males, the population will decrease.

Preparation for the introduction of genetically modified (GM) mosquitoes in dengue:

The advent of an effective of GM in dengue is still some time in the future, and the intervening period would be well used to consider several important elements relating to its successful introduction. A unified political attitude will be essential to enable the control of dengue in the Latin American region. This will entail a clear understanding of the value of, and application of a GM in dengue. In turn, this means the establishment of effective disease surveillance systems and laboratory networks allowing the derivation of benchmark indicators by which to measure the effects of the GM technology. The age of at-risk groups based on serological profiles should be defined, as well as the genetic technology strategies and target areas for its use. In parallel, individual countries must anticipate their regulatory requirements at a national level. However, the development of ad hoc decision making bodies may be necessary, since delays in implementation of GM will

inevitably lead to loss of life due to the disease. A clear educational programme to explain the need for Genetic modification should therefore be made available to all those involved in its implementation. An equally broad scope should be adopted in terms of financing those programmes, conceivably crossing national borders within the region so as to overcome individual countries' monetary shortcomings.

GM Trials in world

This should result in the decrease of *Aedes aegypti* mosquitoes, and in the decrease of dengue transmission. The Brazilian National Biosafety Technical Committee (CTN Bio) approved the method, and a poll conducted in Juazeiro by Malavasi and his team found 90 per cent of residents in favor of the experiment[19]. The first trial, which was conducted in 2009, introduced the modified mosquitoes equivalent to 16% of the population in the Grand Cayman study area, and the fluorescent marker was found in 10% of the larvae, demonstrating that the mosquitoes were effective sexual competitors, although not quite as competitive of their normal counterparts. Another successful field study was conducted; unpublished trial in 2010 saw an 80 % reduction of mosquito population in the target area on Grand Cayman Island. These trials show much promise compared to previous methods such as using radiation to sterilize the mosquitoes, which reduced the mosquitoes' ability to compete with wild males. Malaysia has released 6,000 genetically modified mosquitoes into a forest in the first research of its kind in Asia aimed at to the limitation dengue fever[20]. Brazil is the third country that conducted a suppression trial involving the large-scale release of the Oxitecstrain of *Aedes aegypti*mosquitoes. The project was carried out by the University of Sao Paulo and permission was granted by Brazil's National Biosecurity Technical Commission (CTNBio) in December 2010. Scientists involved in the project reported an overwhelming positive support for the project from all sectors of the community. IMR organized an intensivebiosafety workshop on GM mosquitoes in September 2011 and invited Brazilian scientists to share firsthand information on the public engagement process. The IMR is working closely with regulators and stakeholders towards effective community engagement from lessons learnt in the open field release in the uninhabited site. Although approval with terms and conditions included release for a limited (Marked release and recapture) MRR trial in an inhabited site, the medical research has improved, effective communication plan can be implemented. The communication plan proposed would adopt a pro active approach before any trial begins with advertorials, engagement of journalists, media interview and distribution of flyers from door to door at the trial site. This will promise to more systematic and coordinated public relationship and communication plan for any future releases involving GM mosquitoes. In no case did the company correctly follow this notification procedure, with the result that there was no independent scrutiny of whether these risk assessments met the required European standards.

The fact remains that due to the complexity of this process, we do not have a clear mechanism or guideline for effective communication and outreach especially for health-related projects. This is further complicated by negative perceptions in some quarters over the use of GM technology. The development of effective communication messages should explain the risk and benefit of the GM technology for interruption of dengue transmission and communicate findings of the field trial to the public. Various prevention strategies were used by vector-borne disease control authorities to reduce mosquito burden but despite these strategies the alarming rise of dengue cases and mortality remains a major problem. In the public health context, therefore, risk benefit analysis should form an integral part of the GM regulatory framework [15]. If the scientific evidence from the risk benefit analysis demonstrates an expectation of significant disease reduction with low ecological risks, the precautionary principle in the GM regulatory frame work should not impede meaningful benefits for human health. However if human value cannot be determined or ecological risks are high, caution should be exercised in the approval process of future releases[16]. The themes trans genesis causes numerous reactions in different classes of the human population, and generally genetically modified organisms face strong skepticism. This is the reason to present sufficient information to the public regarding the use of this technology, as public acceptance is a priority and essential for any tests with transgenic mosquitoes in the field and these tests must comply with the requirements of the authorities[21, 22].

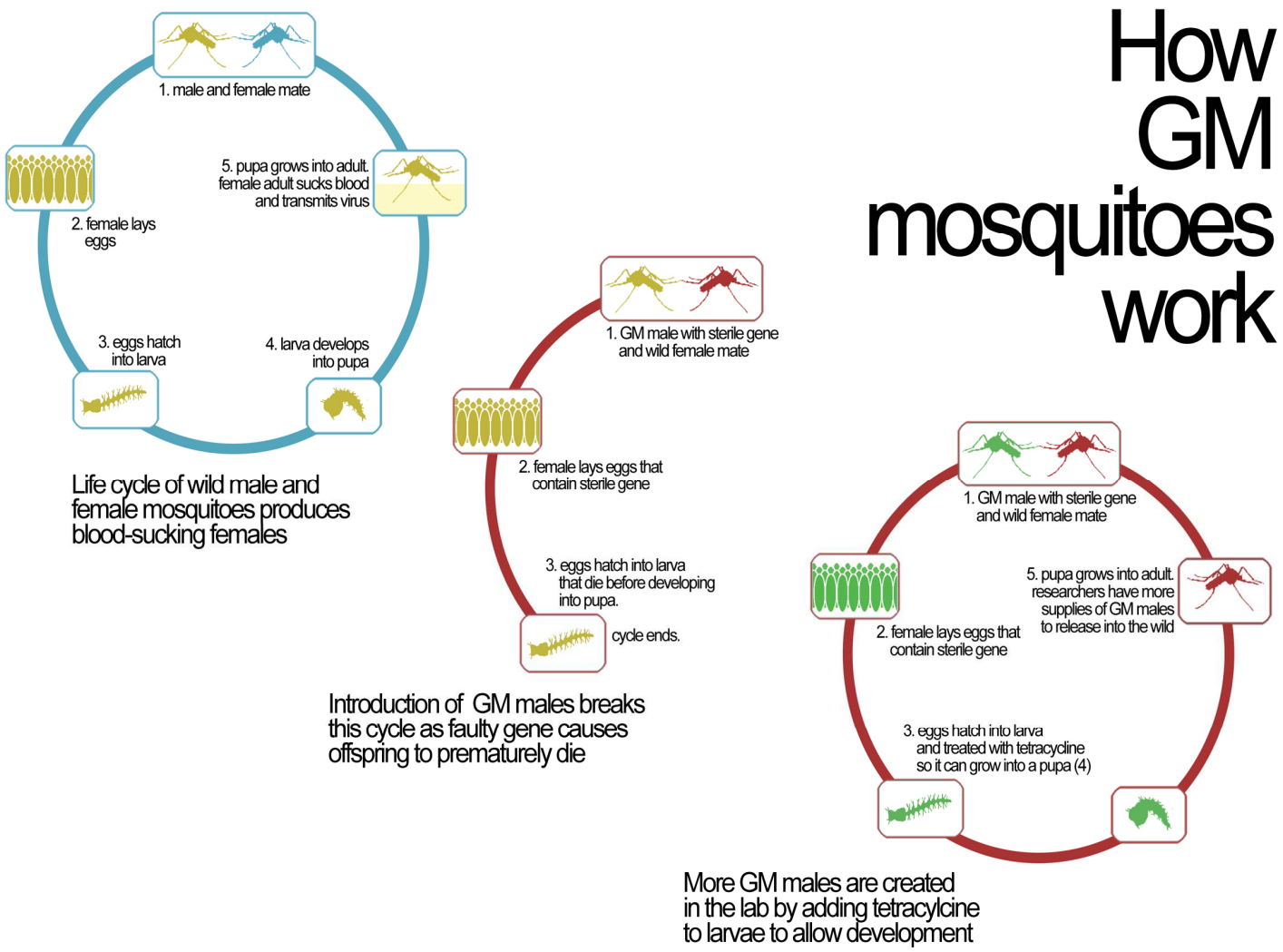

Figure No.1: Breaking the cycle: how the process of releasing GM mosquitoes works

CONCLUSION

Dengue cases are increasing with each passing year in the populations of the world causing unpleasant symptoms in humans preventing them from performing their normal daily activities. Various methods are employed to help control dengue as the use of larvicides and insecticides, but that only minimize dengue cases, their effectiveness is not complete, they cause resistance. Vaccines are being researched and tested, but not yet proven anything. But thanks to the advancement of science by the techniques of molecular biology and genetic engineering, all the latest in the world of scientific research and through these techniques, scientists have studied and came to the conclusion of the innovative use of transgenic mosquitoes that alter the mosquitoes DNA and it has demonstrated better efficacy to date. One issue that needs to be dealt with is keeping any females from being released; unlike males, females bite humans, and the effect of a genetically engineered mosquito's bite on humans is unknown. Future trials will hopefully show promise of genetically modified insects to eliminate all insect-borne diseases, including dengue fever and malaria.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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