Mosquito-borne dengue fever threatens Brazil World Cup but GM solution blocked by biotech fears

The World Cup, hosted this year by Brazil, begins next week. While millions of fans are looking forward to this global event, Brazilian authorities are scrambling to get the venues finished in time for opening kickoffs. But a less publicized crisis looms: the threat of mosquito-borne dengue fever, whose transmission has been significant in Sao Paolo this year.

Over 2.5 billion people – over 40% of the world's population – are now at risk from dengue. The World Health Organization estimates there may be 50–100 million dengue infections worldwide every year.

In this article, I will share my family's experience with dengue fever and one company's approach to combat the disease using genetically modified mosquitoes.

My losing battle with diseased mosquitoes

When I was 6 years old, my family moved from Canada to Venezuela. I graduated from high school there and then chose to move back to Canada to go to college while my family stayed behind. It's only in the past 2-4 years that my family left Venezuela due to the economic and political instability.

Most of that time was spent in a city called Barquisimeto. Our house overlooked a valley of sugar cane fields, called "Valle del Turbio", named for a river that ran close to our house. The river, which translates to "murky", was aptly named because during dry season it was stagnant and stinky. As such, mosquitoes and the illnesses they bear were very common in our neighborhood.

I first learned about dengue fever when I was in 5th grade. It was introduced in health class along with other common tropical diseases such as yellow fever and chagas disease. We learned that it was a mosquito borne illness for which there was no vaccine. The dengue virus is transmitted primarily through the *Aedes aegypti* mosquito and recent statistics suggest that as many as 400 million people are infected each year.

My earliest memory of someone getting dengue was probably in 7th or 8th grade. My dad got dengue, and I very vividly remember seeing him wrapped up in one of the very few blankets we owned, because it never got cold enough for blankets. My dad was lying on the sofa in front of the TV while shivering in the blistering afternoon heat. I yelled for my sister to come see because he was rambling and uttering gibberish, which I thought was hilarious. Years later, I realized that it was due to the very high fever that accompanies dengue, and understood why my mom had been so anxious and worried.

We always took precautions when there were a lot of mosquitoes: all our beds had mosquito netting hanging from hammock hooks in the walls; about one hour before going to bed, my dad would spray all our bedrooms with Baygon bug spray; we used plenty of insect repellent when we sat outdoors; and we never chased away the bats that lived in our mango trees, in hopes that they'd eat their share of mosquitoes at dusk. When one of us had dengue or knew one our neighbors had it, we'd use even more

bug repellent since a person who is infected with the virus can infect a mosquito when they get bitten. In the course of doing research for this piece, I've learned that most of these measures didn't matter anyway, because the mosquito that carries dengue is most active during the day.

I got dengue when I was in 9th grade. I'm the only person in my family who got it only once, probably because I lived in Venezuela for the least amount of time. I remember that my body hurt a lot, like severe growing pains. We never went to the doctor when we got dengue, but recognized it due to the pain that it caused, the high fevers, and the red dots under our skin. Even if we had gone to the doctor, there wasn't anything that could be done, so we just took Tylenol to reduce the fever and rested.

Almost no one in tropical or subtropical regions can elude dengue

My first true scare with dengue happened when I was in my first year of college in Canada. My mom called to tell me that they had taken my sister to a clinic because she had hemorrhagic dengue. I don't think I can accurately describe the fear that this statement caused unless I give you an idea of the status of Venezuela's hospitals. A hospital in Venezuela is just a concrete building where doctors work. That's it. There are no amenities: you have to buy your own medicine, your own sheets, there are long lines to be seen, it's hot, it's disorganized and it's noisy. So we never went to the hospital. Ever. The only time I ever went to a hospital was to visit my brother, who's a surgeon, and it was not an experience that I'd care to repeat.

I once asked my sister-in-law (who is an Ob/Gyn) what had been her craziest experience in the hospital. She and my brother worked in our state's largest hospital for several years. She said that in the middle of a C-section, there had been a power outage and, of course, there was no emergency generator. So she called all her students into the OR and asked them to turn on the lights on their cell phones, and she finished her surgery to the glow of Blackberry's and iPhones. So based on that single phrase, "we've taken your sister to the clinic", I knew just how bad it had to be.

Dengue causes platelet and white blood cell counts to drop, leading to an inability to clot blood and internal bleeding. Patients then go into shock and can die within 24 hrs. According to the WHO, dengue has a fairly low mortality rate: 2.5%/ Yet most of those who are hospitalized are young children. While doctors were deciding whether my sister needed a blood transfusion, they noted that her platelet count was rising and she was able to recover within a few days. She wrote to me about her experience: "The nasty part of dengue was the weakness, feeling dizzy and faint all the time. When I was in the hospital I remember getting nosebleeds a lot by just touching or scratching my nose a little. That's how low my platelet levels were."

I asked my family members to describe their own experiences with dengue. My dad said: "I had it 2 or 3 times. The things I remember are high fever, headache, pain in joints and bones, general weakness and discomfort which would last for a relatively long time and take time to recuperate, skin rash in case of hemorrhagic dengue, and low platelets that may result in blood transfusion. In Venezuela they give you folic acid to increase platelets, a pain killer that is not Aspirin, and lots of liquid."

My brother wanted me to stress a few other points, particularly insecticide use in public areas during

epidemics: DDT was used in Venezuela until fairly recently. He mentioned that Abate larvicide was used in the drinking water in places where people didn't have running water. Although it's WHO approved for drinking water, he thought "it makes it taste horrible." Finally, my sister-in-law mentioned that she remembers having patients who miscarried during dengue epidemics.

GM mosquitoes demonstrated to combat dengue have been developed

You can imagine that I'm pretty biased and was elated to learn that a genetically modified mosquito has been approved to combat dengue in Brazil. Although the mortality rate for dengue may not be high, it carries billions of dollars in costs to individuals and health care when there is an epidemic.

The GM strain of mosquito (OX513A) has been developed by a British biotech company called Oxitec. Their webpage has a pretty simple description of the technology: the genetically modified mosquitoes need a specific chemical, tetracycline, to survive. If they do not have access to tetracycline, they die. The modified mosquitoes are released into the environment where they mate with wild-type mosquitoes, and the modified gene is passed on to their offspring. The modified mosquitoes and all of their offspring die, because they will not have access to the supplement.

The technology is called RIDL (Release of Insects carrying a Dominant Lethal gene). The use of sterile mosquitoes to control population is not a new strategy: SIT (Sterile Insect Technique) has been used for several decades, but since the sterility is induced through radiation, the insects are weakened, causing " reduction of the longevity, sexual vigor and general activity of males". Therefore, the use of genetically modified mosquitoes could be considered an improvement over SIT.

There are already several papers published on the OX513A mosquito, and as far as I could tell, all or most of the studies were (understandably) written in collaboration with Oxitec. OX513A requires tetracycline in order to survive. In the absence of tetracycline, its offspring die at late larval or early pupal stage (details of the mechanism of action of the transgene can be found here).

This antibiotic can be easily provided to the skeeters in the laboratory, but would be difficult for them to find in the wild. This was an important point for me, because as a fan of Jurassic Park, I knew that if dinosaurs were able to escape and find a source of lysine, then it might be possible for mosquitoes to find a source of food required for their survival. However, unlike lysine, which is found everywhere, the antibiotic is made synthetically so it is almost impossible that the mosquitoes would ever find an abundant source of tetracycline.

The modification for OX513A is non-sex-specific and the mosquito also carries a red-fluorescence protein for visual identification (wouldn't it be AWESOME to see a glowing mosquito when you hear that annoying buzzing sound in the middle of the night right next to your ear!?!). For the purposes of the control program, only male RIDL mosquitoes will be released into the wild.

A study in 2011 compared lifespan and other metrics between the genetically modified mosquito and its wild-counterpart. It found that "unmodified mosquitoes survived on average about 5% better than the transformed OX513A line", and the unmodified mosquitoes pupated on average one day later than the

modified ones. The unmodified mosquitoes were also larger and lived longer, concluding that there are statistically significant differences between OX513A and its wild-counterpart.

The authors suggest several hypotheses for their findings: the silencing of the lethal gene may not be complete; the transgene may have negative effects; the transgene may have inserted itself in a region where it is impacting surrounding genes' or that the strain of mosquito is too inbred and may be expressing recessive mutations. The authors conclude that it will be necessary to determine if any of the differences observed in the OX513 mosquitoes have an impact on mating capacity (i.e. will 'normals' still find the mutants "sexy"? And unfortunately, a more rigorous study would be required than gauging audience reactions to Mystique in X-Men).

The question of mating capacity is key to the success of this entire program. I was surprised to learn that the *Aedes aegypti* female is monogamous, while the male is polygamous. So if the wild-type female were to prefer the wild-type male, then the whole project would fall apart because the mutant gene would never pass on to the next generation. A study examining mating capacity was published late 2011, and compared OX513A with it's closest wild relative. Wild-type males lived longer and inseminated more females than OX513A. The authors found that the wild-type and mutant males initially mate with the same number of females, suggesting that males of both strains may have the same amount of initial sperm and energy reserves, but the mutants don't regenerate their capacity as easily. They say this means OX513A would have to be released into the wild more frequently and that it doesn't exclude their use in a control program.

I don't know how Oxitec prices their program, but if it is by the number of mosquitoes required, then this is a pretty convenient problem to have. Instead of requiring the release of males every X number of weeks/days, the findings of this paper suggest that the releases would have to be done more frequently than expected. Just to be clear, I'm speculating on the fact that this translates into greater costs to the consumer since the company's website was vague on the cost of their program.

Another important topic was the impact of the mutant mosquito on its predators, that is to say, how do the mutant proteins impact the animals who eat the mutant mosquitoes? In a paper published last year, the authors used the 2 species of the predatory *Toxorhynchites* (known as the "elephant mosquito") to answer this question. These large mosquitoes eat the larvae of other mosquitoes, including *Aedes aegypti.* The scientists' choice of organism was a smart one: they wanted an organism small enough to be impacted by eating OX513A, that could be studied in the lab, and that could subsist exclusively by eating the mutants (this last point is key to the value of this study). They used 3 different diets: wild-type larvae, OX513A reared with tetracycline, and OX513A reared without tetracycline.

Toxorhynchites females eating wild-type larvae ate more larvae than females eating OX513A larvaereared on tetracycline. The authors have no explanation for this statistically significant difference,particularly since it was not seen in the second species tested (it would be interesting to see if this couldbe reproduced, particularly with more mosquitoes – each treatment group had just ~20-30 mosquitoes forthis study). All other comparisons were either equivalent, or could be explained. Most importantly, therewas no difference in lifespan, development, or fecundity in the predators, and the authors conclude thatthe OX513A mosquitoes are unlikely to impact predators in the environment.

Oxitec also did a field test in the Cayman Islands and its results were published in 2011 in Nature Biotechnology. They wanted to know how the mosquitoes would mate in the wild and whether the patterns they observed in the lab would carry over into the "real world". For their study, "OX513A males were released in a 10-hectare (ha) area at an average release rate of 465 males/ha/week for 4 weeks, starting on Nov. 16, 2009." They set up traps over the course of the study to determine how many of the larvae were mutants. They conclude that the mutant males can compete fairly well for mates in the "real world" (i.e. mutants are sexy!!).

The last paper I read was about the release of male OX513A mosquitoes in Malaysia. The goal of the study was also to determine what happens to the mosquitoes when they are released in the wild, and was carried out in a region where there are no humans. The study, published in 2012, was approved by Malaysian regulatory agencies and was carried out in an uninhabited area. However, many public engagement activities were performed prior to the study's launch. The mosquitoes were released and monitored using nets, and several parameters were measured and compared to a control mosquito species. I thought that this sentence in the discussion was encouraging: "As with previous field releases, the transgene disappeared rapidly from the environment post-release, as expected, and was not detected more than a few hundred metres beyond the release area." Translation: the mutants don't go too far, and they die quickly (as expected).

Predictable anti-GMO opposition

I couldn't find any readily available studies outlining the data from Brazil's trials, but this news article states that the trials have been ongoing for 2 years and have seen a 90% drop in wild *Aedes aegypti.* As expected, the article also outlines opposition from environmental groups, whose concerns are:

- *A small number of [mutant] female mosquitoes could also be released – and end up biting people.* My perspective? At least the OX513A female mosquitoes don't carry dengue. Given the choice between getting bitten by a mutant mosquito vs a dengue carrying mosquito, bring on the mutants.

- *Some of the new offspring could survive by feeding on food or waste contaminated with tetracycline and therefore pass on their GM traits.* Seriously? How would this happen? I'm not sure how food or waste could get contaminated with enough tetracycline so that the mosquitoes get their "fix"? Tetracycline was discovered from a soil dwelling bacteria, and the antibiotic is abundantly produced via fermentation in the lab. However, it is within the realm of possibility that a cargo truck carrying tetracycline might topple over, thereby creating pools of antibiotics that the mosquitoes could then

lap up. I'll give you that.

There is additional outrage in the United States because this technology was contemplated as a possible tool to combat dengue in the Florida Keys. According to the article that I've cited, there are allegations that the study in the Cayman Islands was done secretively, although Oxitec rebuts this claim on their website. In any case, the company probably learned its lesson, and seems to have done more visible community engagement activities in Malaysia and there are reports of the same being done in Brazil and Panama.

The story from the Florida Keys states that concerned citizens feel that not enough independent research has been done. Personally, I feel that there's quite a bit published already and just because there are Oxitec scientists among the researchers on a study should not automatically invalidate its findings. The papers that I cited above were research projects led by major universities and to omit Oxitec entirely from the list of authors would not make sense, since the product belongs to Oxitec and has not yet been commercialized.

In the comments section on the news story from the Florida Keys, there was concern that the mutant gene might harm humans. Again, I don't see a logical mechanism how this may happen. The dengue virus lives on the mosquito's salivary glands, which is how it gets transmitted when we get bitten. But I couldn't find any information on mosquito cells getting transferred in the biting process. However, let's assume that mosquito cells *do* get transmitted to humans when we get bitten. It would imply that mosquito cells have been getting transmitted to humans for thousands of years while humans and mosquitoes have co-existed. The two proteins that are being expressed in OX513A mosquitoes would not cause any change in what is already happening when we get bitten.

An additional concern is the possibility that the mosquitoes will be released into the wild with no regulation. I don't think this concern is valid, based on several reasons: a) If a company releases genetically modified insects secretly into the wild, then how do they make money? b) Genetically modified living organisms are governed by the Cartagena Protocol on Biosafety, which is an "international agreement which aims to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into account risks to human health." As such, Oxitec has been working closely with public health organizations in each country who, after all, are their customers.

My perspective is that this company has done the legwork: they started with a few publications outlining their findings, moved on to field trials where there were no inhabitants, and finally to trials where there were humans. It seems that they've done all the logical tests (note that there were several other studies on these mosquitoes that I didn't cover in this blog post). I think that the only research that I'd be interested in seeing is whole genome sequencing of the mutant to determine if a root cause for the short lifespan can be identified, but that is more out of scientific curiosity. I don't think we can say that this is a technology that has not been tested: thousands of mosquitoes representing hundreds of generations have been tested throughout the course of the last decade, and it seems that everything is fine.

So my vote is for this technology to be adopted worldwide. During her review of this piece, my sister took it one step further: "I got an advisory from the Canadian embassy in Venezuela on a Chikunguya outbreak

in Latin America. I assume the technology could be applied to this disease, too.”

I think my whole family would agree on one thing: we recommend its test in the ”Colinas del Turbio” neighbourhood in Barquisimeto, Venezuela

Layla Katiraee, contributor to the Genetic Literacy Project, holds a PhD in Molecular Genetics from the University of Toronto and is a Senior Scientist in Product Development at a biotech company in California. All opinions and views expressed are her own. This article also appeared in biofortified.org. Her twitter handle is: @BioChicaGMO.