eunoia
JUNIOR COLLEGE

Impact of Climate Change on Plants, Animals & Man (III)

1. Introduction

Mosquitoes and their itchy bites are more than just an annoyance. They transmit dangerous viruses with deadly consequences – making them the most lethal animal on Earth. It's the *Aedes* mosquito species that are behind outbreaks of dengue virus, Zika virus, yellow fever virus and Chikungunya virus.

Approximately 390 million dengue infections occur every year, with as many as 96 million resulting in illness. Dengue fever is widespread in Singapore and in the region of Southeast Asia. The prevalence of the virus is closely tied to the prevalence of the *Aedes* mosquito, the transmitter of the infection. Despite five decades of battling dengue, the fight in Singapore remains a Sisyphean task.

2. Learning Outcomes

(f) Outline the life cycle of *Aedes aegypti* as an example of a typical mosquito vector.

(g) Outline the development of viral dengue disease in humans, including host-pathogen interactions, human susceptibility to the virus; pathogen virulence, transmission and drug resistance.

Contents

3. Mosquito Vector & Dengue

Notes to self

The main vector of dengue virus is the mosquito *Ae. aegypti*, as dengue virus is transmitted between humans through the bite of infected female *Ae. aegypti* mosquitoes.

Dengue cannot be spread directly from one person to another, and mosquitoes are necessary for the transmission of the dengue virus. The dengue virus is thus spread through a human-to-mosquito-to-human cycle of transmission (Fig. 1). Infected mosquitoes can continue transmitting the dengue virus to healthy people for the rest of their life spans, generally a two to three week period under natural conditions.

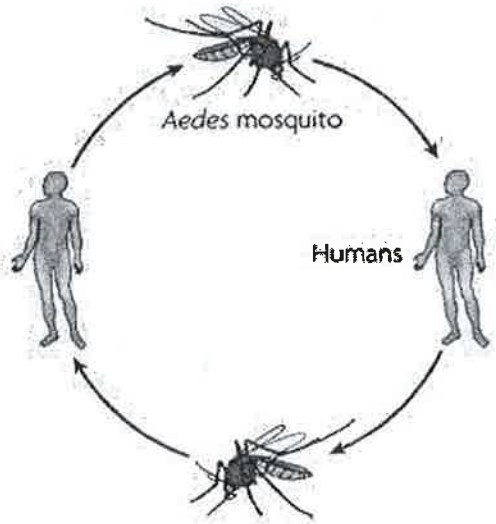


Fig. 1: Dengue transmission.

Dengue poses the greatest risk in highly populated regions with rainy seasons where there are large populations of *Ae. aegypti* with a high degree of contact between the mosquitoes and humans.

a. Life Cycle of *Aedes aegypti* Mosquito

Notes to self

Ae. aegypti is a holometabolous insect, meaning that it goes through a complete metamorphosis with an **egg**, **larva**, **pupa**, and **adult stage**. The adult life span can range from two weeks to a month depending on environmental conditions.

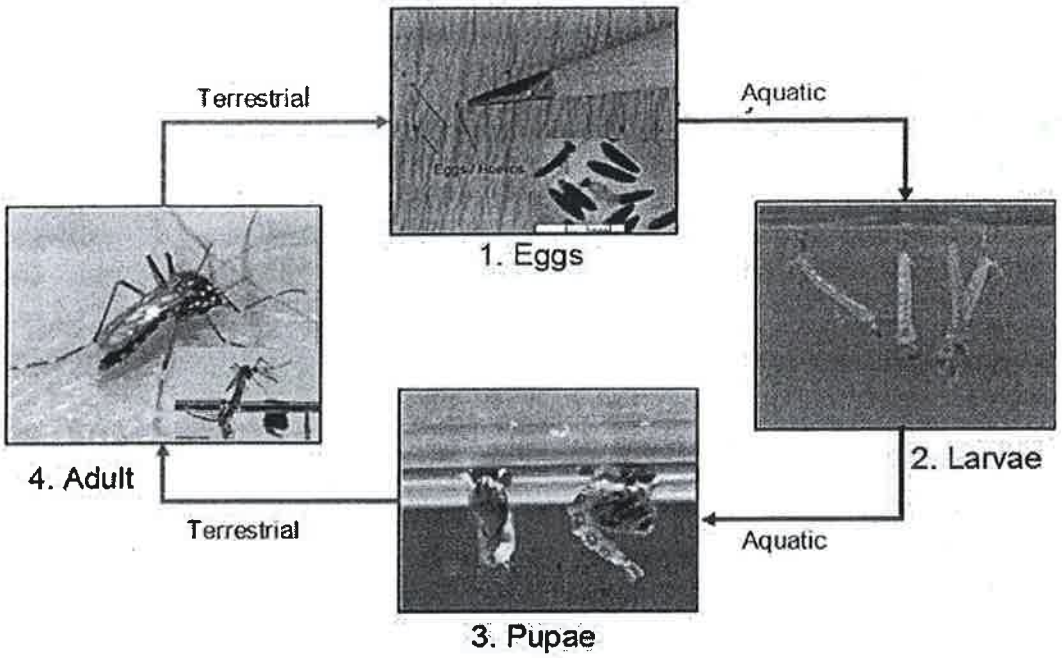


Fig. 2: **Life cycle of *Ae. aegypti*.** Female mosquitoes lay their eggs on the inner, wet walls of containers with water. Larvae hatch from eggs (picture 1, inset) when water inundates the eggs as a result of rains or the addition of water by people. In the following days, the larvae (picture 2) will feed on microorganisms and particulate organic matter, shedding their skins three times to be able to grow from first to fourth instars (not shown in diagram; an instar refers to the developmental stage of an arthropod, e.g. insects, between moults until sexual maturity is reached). When the larva has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa (picture 3). Pupae do not feed; they just change in form until the body of the adult, flying mosquito is formed. Then, the newly formed adult emerges from the water after breaking the pupal skin (picture 4, inset). The entire life cycle lasts 8-10 days at room temperature, depending on the level of feeding. Thus, there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) in the *Ae. aegypti* life cycle.

Egg stage

Notes to self

After taking a blood meal, the female *Ae. aegypti* mosquito produce on average 100 to 200 eggs per batch. The female can produce up to five batches on eggs during a lifetime. The number of eggs is dependent on the size of the blood meal.

Eggs are laid on damp surfaces in areas likely to temporarily flood (e.g. tree holes and man-made containers like barrels, drums, jars, pots, buckets, vases, tins, tyres, discarded bottles etc). Eggs will most often be placed at varying distances above the water line.

In **warm climates**, **eggs may develop**, whereas in cooler temperature climates, development is slower and can take up to a week.

Laid eggs can **survive for very long periods in a dry / dessicated state**, often for more than a year. However, they **hatch within one to two days in water.** This means that even if all larvae, pupae, and adults were eliminated at some point in time, re-population will occur as soon as the eggs in the containers are flooded with water. This makes the control of the *Ae. aegypti* mosquito a very difficult task.

Fig. 3: *Ae. aegypti* eggs. ~1mm long. Smooth, long and ovoid shaped.

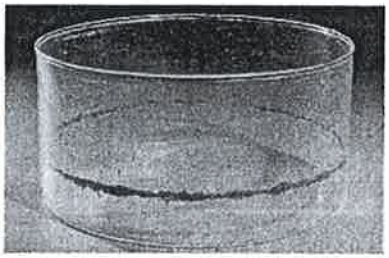


Fig. 4: *Ae. aegypti* eggs laid above water line.

Larval stage

After the eggs hatch, the larvae feed on organic particulate matter in the water, such as algae and other microscopic organisms. Most of the larval stage is spent at the water surface, although they will swim to the bottom of the container if disturbed or when feeding. *Ae. aegypti* larvae **breathe oxygen through** a posteriorly located **siphon**, which is **held above the water surface** while the rest of the body hangs vertically.

Larval development is temperature dependent. The larvae passes through **four instars / larval stages**, spending a short amount of time in the first three, and up to three days in the fourth instar (**Fig. 6**). Fourth instar larvae are approximately 8mm long. If temperatures are cool, *Ae. aegypti* can remain in the larval stage for months so long as the water supply is sufficient. The larva generally takes about **four days** to develop into a pupa.

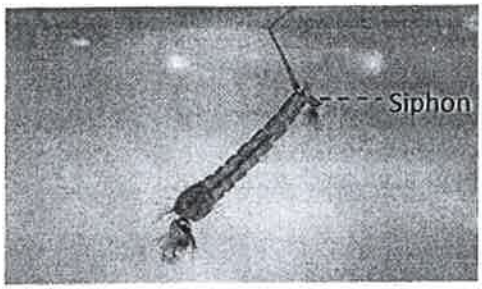


Fig. 5: Larvae of *Ae. aegypti.*

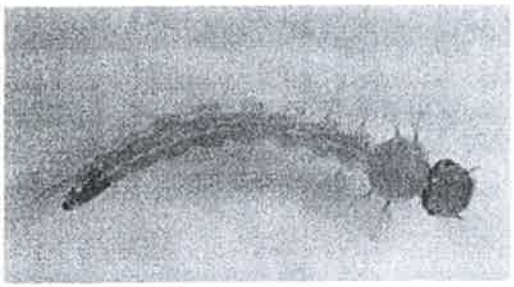


Fig. 6: Fourth instar larvae of *Ae. aegypti.*

Notes to self

Pupal stage

After the fourth instar, the larvae enters the pupal stage. Like the larval stage, pupal stage is also **aquatic**.

Pupae do not feed and take approximately **two days** to develop into a fully developed adult mosquito.

↓ Extrinsic incubation period
time taken for DENV to replicate & disseminate in mosquito
Mosquito becomes infectious for longer.
Higher chance of infecting a person before it dies!

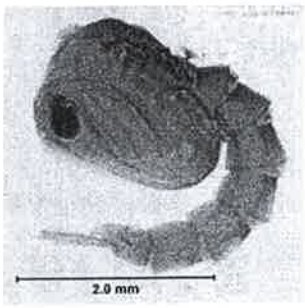


Fig. 7: Pupae stage.

Adult stage

Adults emerge from pupal stage after two days by **ingesting air** to **expand the abdomen**, thus **splitting open the pupal case** and **emerging head first**.

Three days after the female mosquito has taken a **blood meal**, it will **lay eggs** and the **cycle begins again**.

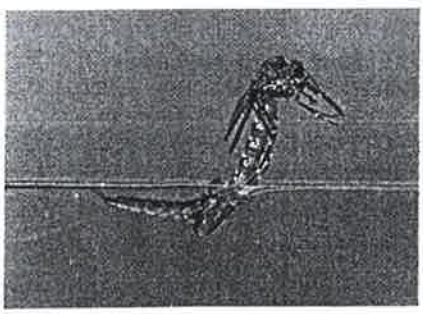


Fig. 8: Emerging adult.

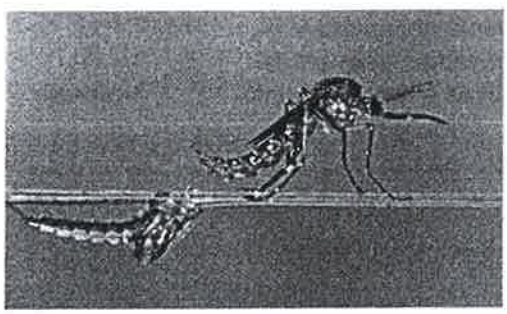


Fig. 9: Emerged adult.

b. Development of Dengue in Humans

Notes to self

When an infected mosquito feeds on a person, it injects the dengue virus into the bloodstream. The virus infects nearby skin cells called keratinocytes, the most common cell type in the skin. The dengue virus also infects and replicates inside Langerhans cells.

Once the Langerhans cells are infected with the dengue virus, they travel from the infection site in the skin to the lymph nodes and display dengue viral antigens on their surface.

The displayed antigens activate the innate immune response by alerting two types of white blood cells, monocytes and macrophages, to fight the virus. Normally, monocytes and macrophages ingest and destroy pathogens, but instead of destroying the dengue virus, both types of white blood cells are targeted and infected by the virus.

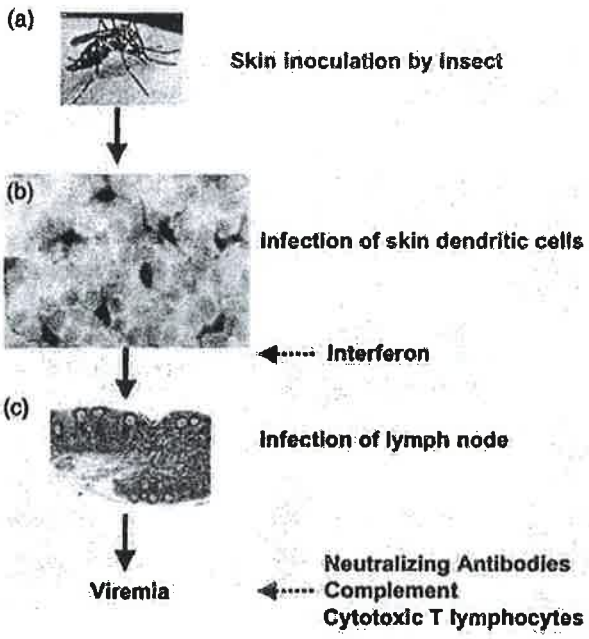
As the infected monocytes and macrophages travel through the lymphatic system, the dengue virus spreads throughout the body and infects more cells. These include cells in the lymph nodes and bone marrow, macrophages in both the spleen and liver, and monocytes in the blood. The spread and increase of the virus results in viremia, a condition in which there is a high level of dengue virus in the bloodstream.

Fig. 10: Dengue Viral Infection

(a) A person is infected with the dengue virus when an infected mosquito bites the person's skin.

(b) The dengue virus infects the Langerhans cells, a type of dendritic cell in the skin.

(c) The infected Langerhans cells produce interferons to help limit the continuous spread of the infection. Other infected Langerhans cells travel to the lymph nodes carrying viruses, which infect more cells. The spread of the dengue virus results in viremia, which is a high level of the virus in the bloodstream. To fight the infection, the immune system produces antibodies to neutralize the dengue viral particles, and the complement system is activated to help the antibodies and white blood cells remove the virus. The immune response also includes cytotoxic T cells (lymphocytes), which recognize and kill infected cells.



Langerhans cell: a type of dendritic cell. Langerhans cells detect invading pathogens and display antigens from the pathogens on their surface. These cells then migrate to the lymph nodes and alert the immune system to trigger the immune response because a pathogen is in the body.

How does the immune system defeat the dengue virus?

Notes to self

Despite infecting immune cells and spreading throughout the body, the immune system has additional defenses to fight the virus.

The infected cells produce and release small proteins called interferons that are part of a large group of proteins called cytokines. Interferons have the ability to interfere with viral replication, and they activate both the innate and adaptive immune system defenses. They help the immune system recognize dengue-infected cells and help protect uninfected cells from infection. As the immune system fights the dengue infection, the person experiences a fever.

As the adaptive immune response starts fighting the dengue infection, B cells produce antibodies called IgM and IgG that are released in the blood and lymph fluid, where they specifically recognize and neutralize the dengue viral particles.

In another adaptive immune response, cytotoxic T cells, or killer T cells, recognize and kill the cells that are infected with the dengue virus.

The innate immune response activates the complement system, a response that helps the antibodies and white blood cells remove the virus. Together, the innate and adaptive immune responses neutralize the dengue infection, and the patient recovers from dengue fever.

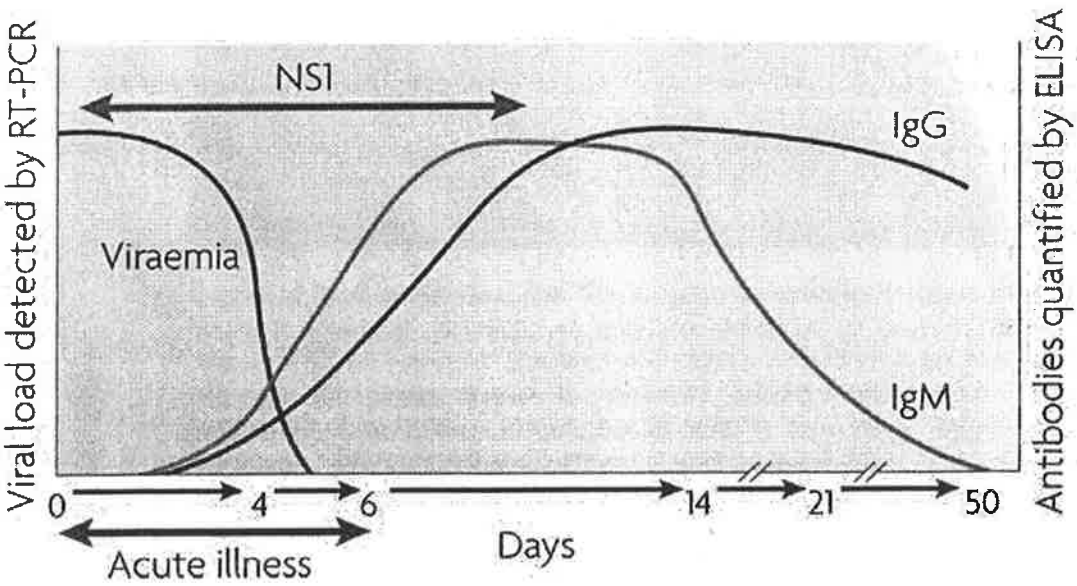


Fig. 11: Immune Response to Dengue Infection
An infected person experiences the acute symptoms of dengue when there is a high level of the virus in the bloodstream. As the immune response fights the dengue infection, the person's B cells begin producing IgM and IgG antibodies that are released in the blood and lymph fluid, where they recognize and neutralize the dengue virus and viral molecules. The immune response eliminates the virus, leading to recovery.

Secondary Dengue Infections

Notes to self

After recovering from a first dengue infection, a person is still susceptible to future dengue infections. This is because there are four different types of dengue viruses (serotypes), but the memory cells only provide immunity from re-infection with the dengue serotype that caused the first infection.

Severe dengue can occur when a person who has developed immunity to one strain of dengue virus becomes infected with another strain. The more lethal forms of Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome, have been observed to occur frequently with secondary dengue infection.

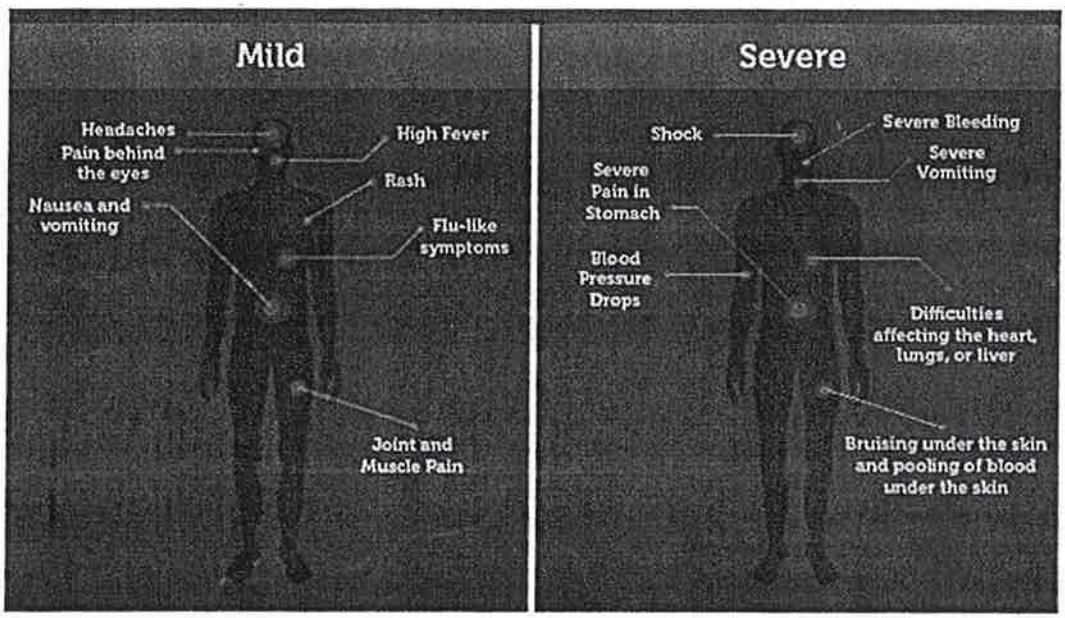


Fig. 12: Mild and Severe Symptoms of Dengue. DF can sometimes lead to severe dengue. DHF is characterised by increased vascular permeability, decrease in blood volume in the body and abnormal blood clotting mechanisms. In moderate DHF cases, all symptoms abate after fever subsides. However, in severe cases, the patient's condition deteriorates after a few days of fever; blood pressure can drop to dangerous levels, causing shock - DSS. DSS is also characterised by bleeding that may appear as tiny spots of blood on the skin and larger patches of blood under the skin. DSS may cause death in 12 to 24 hours.

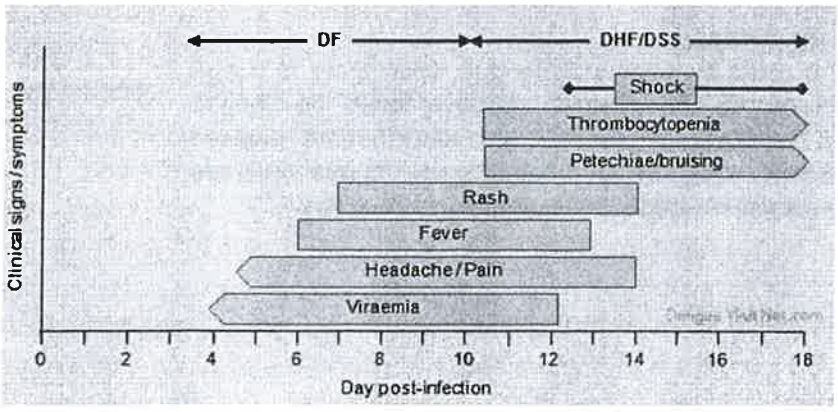


Fig. 13:
Generalised time course of events associated with dengue fever, DHF and DSS.
The incubation period before the development of signs of infections generally ranges from 4 to 7 days.

Notes to self

Severe symptoms of dengue are due to a phenomenon called "antibody-dependent enhancement of infection".

When a person is infected with a second dengue serotype, antibodies from the first infection actually help the virus infect host cells more efficiently, thus increasing viremia.

Ironically, the consequence of antibody-dependent enhancement is that the body's immune system response actually makes the clinical symptoms of dengue worse and raises the risk of severe dengue illnesses.

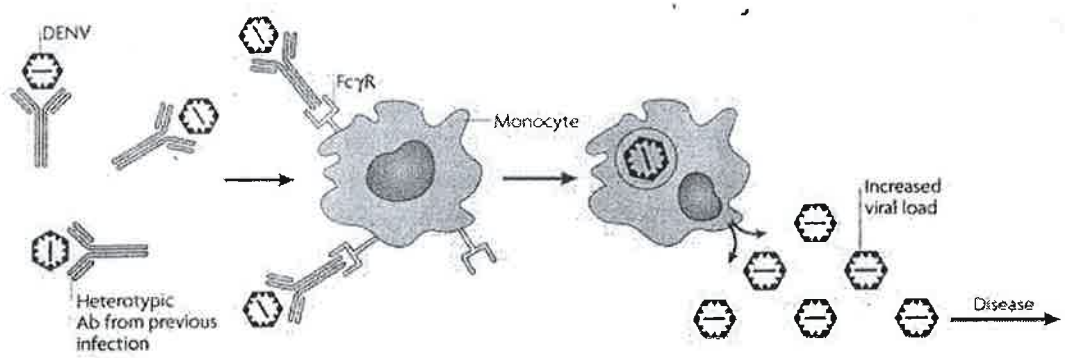


Fig. 14: Model of Antibody-Dependent Enhancement of Dengue Infection.
Antibody (Ab)-dependent enhancement of infection occurs when pre-existing antibodies present in the body from a primary (first) dengue virus (DENV) infection bind to an infecting DENV particle during a subsequent infection with a different dengue serotype. The antibodies from the primary infection cannot neutralize the virus. Instead, the Ab–virus complex attaches to receptors called Fcγ receptors (FcγR) on circulating monocytes. The antibodies help the virus infect monocytes more efficiently. The outcome is an increase in the overall replication of the virus and a higher risk of severe dengue.

Researchers also observed that during a second infection with dengue, the cytotoxic T cells produced by the immune system provide only partial immunity against the new dengue serotype.

The cytotoxic T cells do not effectively clear the virus from the body, and they release excess quantities of molecules called cytokines.

In normal quantities, cytokines help the immune response; however, in high quantities, cytokines can produce serious inflammation and tissue damage such as leakage from the capillaries, possibly contributing to the development of severe dengue diseases.

Notes to self

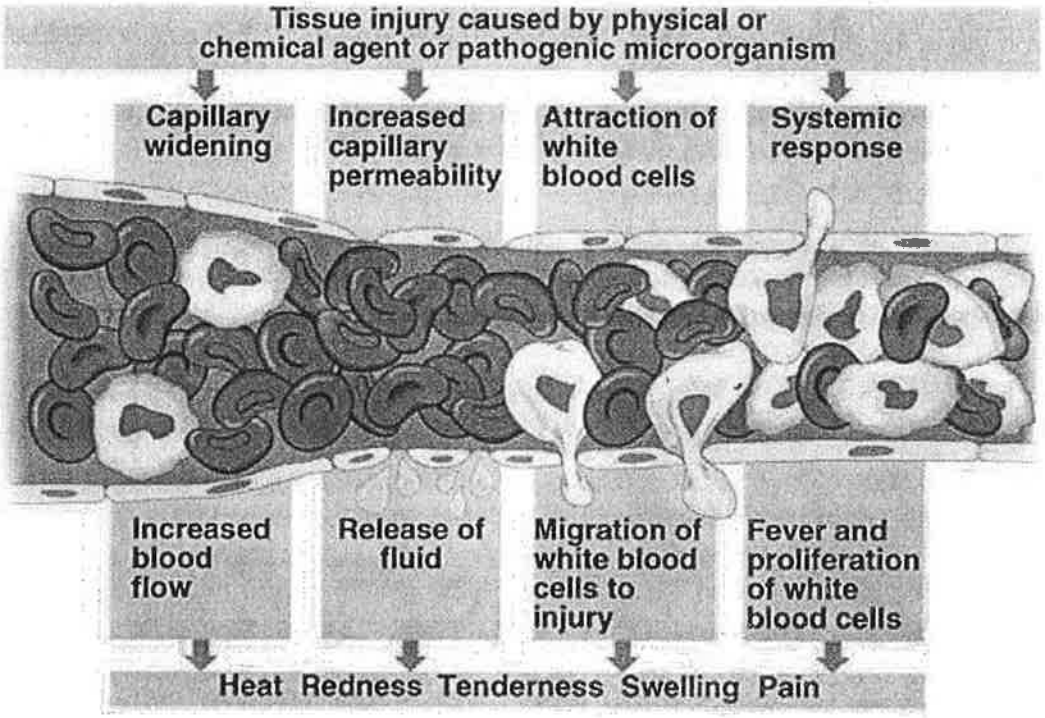


Fig. 15: Characteristics of Inflammation. (The McGraw-Hill Companies, Inc)

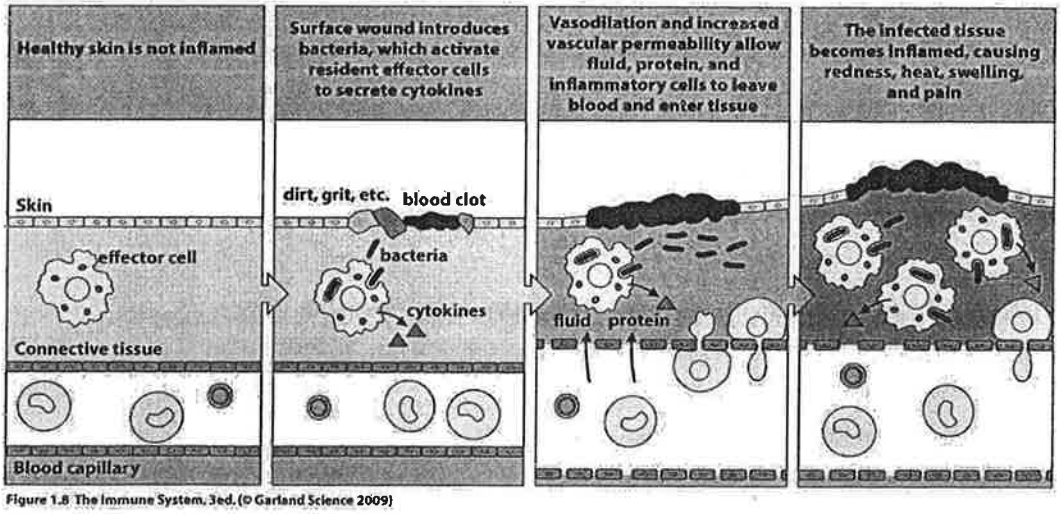


Fig. 16: Role of Inflammation in Immune Response.

The *Wolbachia* Approach to Tackle Dengue

Notes to self

Wolbachia are bacteria naturally found in insects throughout the world. They live inside a host organism's cells. From there, *Wolbachia* are able to manipulate their host in many ways. This wide range of impacts include male killing, feminisation and cytoplasmic incompatibility for instance.

Aedes aegypti mosquitoes don't naturally carry *Wolbachia*. when researchers infected *Aedes aegypti* in the lab, the viruses they carry replicated less. This in turn meant that disease transmission was limited – they were less likely to be passed on when mosquitoes fed on their prey.

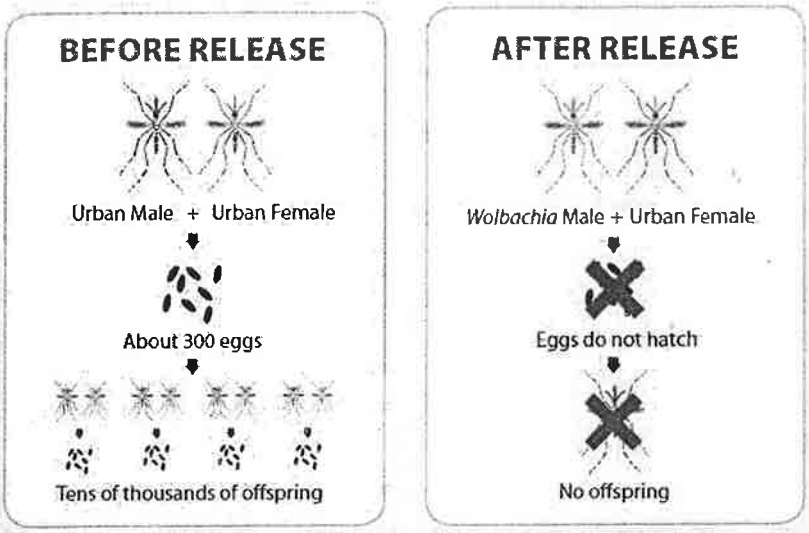


Fig. 17: Using Cytoplasmic Incompatibility to Reduce Mosquito Populations. Cytoplasmic incompatibility occurs when a male mosquito infected with *Wolbachia* mates with an uninfected female. A male mosquito infected with *Wolbachia* has its sperm DNA altered in a way that allows offspring to survive only if the fertilized egg has *Wolbachia*. In addition, since *Wolbachia* is only transmitted through the female egg, the offspring will be *Wolbachia*-free even if *Wolbachia*-infected male mosquitoes are released into the environment. Therefore, the infected males will come in contact only with the naturally occurring *Wolbachia*-free population, and their offspring will die during embryonic development – the eggs won't hatch.

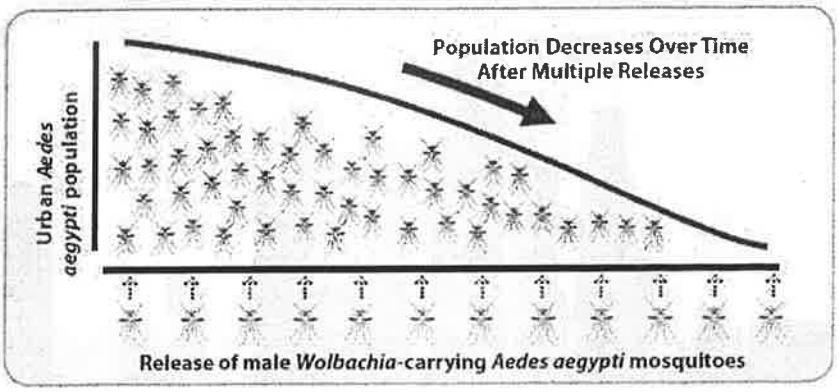


Fig. 18: Release *Wolbachia*-infected males into the environment that will mate with females and cause all offspring to die, eventually leading to a mosquito population crash.