Science Case Study

"Can Bacterial Transformation Stop the Spread of Malaria?"

I. The Global Impact of Malaria

Lerato sits in the clinic, watching her son Baruti, age 4, writhe with fever. He is sleeping for the first time in days, and Lerato is anxious for signs that the medication the doctor had given her son is working. She worries she may have waited too long to bring Baruti to the clinic.

Baruti had fallen ill a week before with fever, chills, and body aches, all vague flu-like symptoms that Lerato had assumed would clear in a few days, as so many illnesses had in the past. Lerato and her family live an hour's walk away from the medical clinic in Seronga, a remote village in Okavango, Botswana. Making that long trip with a sick child is difficult, but it was a trip she had to make when her son's symptoms grew more severe. He was now in a clinic bed, suffering from extreme anemia secondary to (brought on by) malaria.

Malaria is spread by mosquitoes, and like so many others in the Okavanago region, Lerato's family had been issued mosquito nets for their beds. The nets are covered with insecticidal chemicals and are an effective and relatively inexpensive method for controlling mosquitoes. The nets, though, do not allow much air circulation and so are very hot to sleep under, and little Baruti tends to kick them away while he sleeps, exposing his limbs to the bites of mosquitoes.

Questions

1. Malaria is the third leading cause of infectious disease death in the world, after tuberculosis and AIDS. According to the World Health Organization, 3.4 billion people — nearly half the global population — are currently at risk for malaria. Most prevalent in African or tropical Asian countries, malaria is often considered a "disease of the developing world." Though vaccines are not yet available, it can be cured if diagnosed and treated promptly.

   Given this information, what might the biggest hurdles be in fighting malaria? Consider the regions the disease affects and the challenges faced by the people living there.

2. Malaria can be spread only through the bite of a mosquito, and it was nearly eliminated in the U.S. back in the 1950s. Despite this, as many as 1,500–2,000 new cases of malaria are reported in the U.S. annually. How can this be? How might malaria be coming into the country?

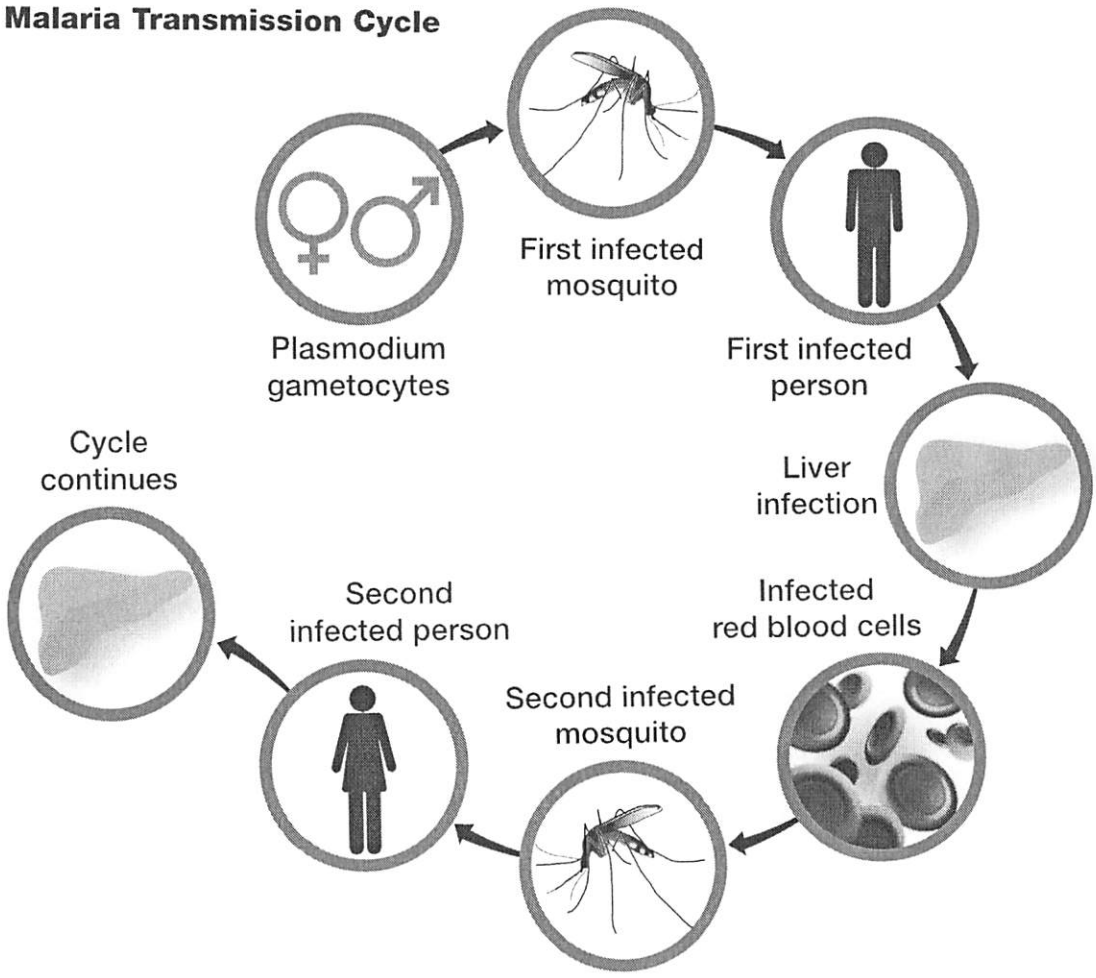
3. Considering that malaria can be spread only from infected blood and through mosquito bites, how might malaria eventually be eradicated in a particular region, such as the U.S.?

II. Mosquitoes — Flying Factories of Malaria

In order to expand the discussion of malaria and possible methods for its treatment, control, and eradication, it is important to understand the biology behind the disease.

Plasmodium — the parasitic protist behind malaria

Malaria is a parasitic infection caused by single-celled protists in the genus *Plasmodium*. Of the more than 100 species of *Plasmodium*, only four infect humans and cause disease. *Plasmodium* is a member of the phylum *Apicomplexa*, a fascinating group of protists believed to have evolved from photosynthetic dinoflagellates (forms of plankton). It has a complex life cycle that will not be discussed here except to say that to complete that life cycle, *Plasmodium* requires two hosts: (1) humans and (2) the female *Anopheles* mosquito.

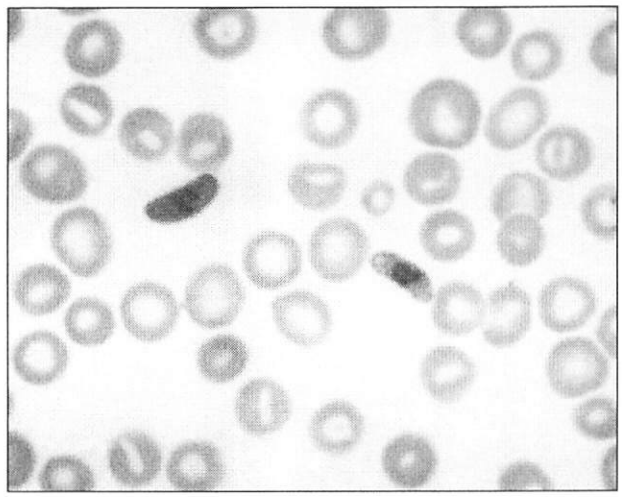


The* Plasmodium *life cycle depends on two hosts: humans and mosquitoes.

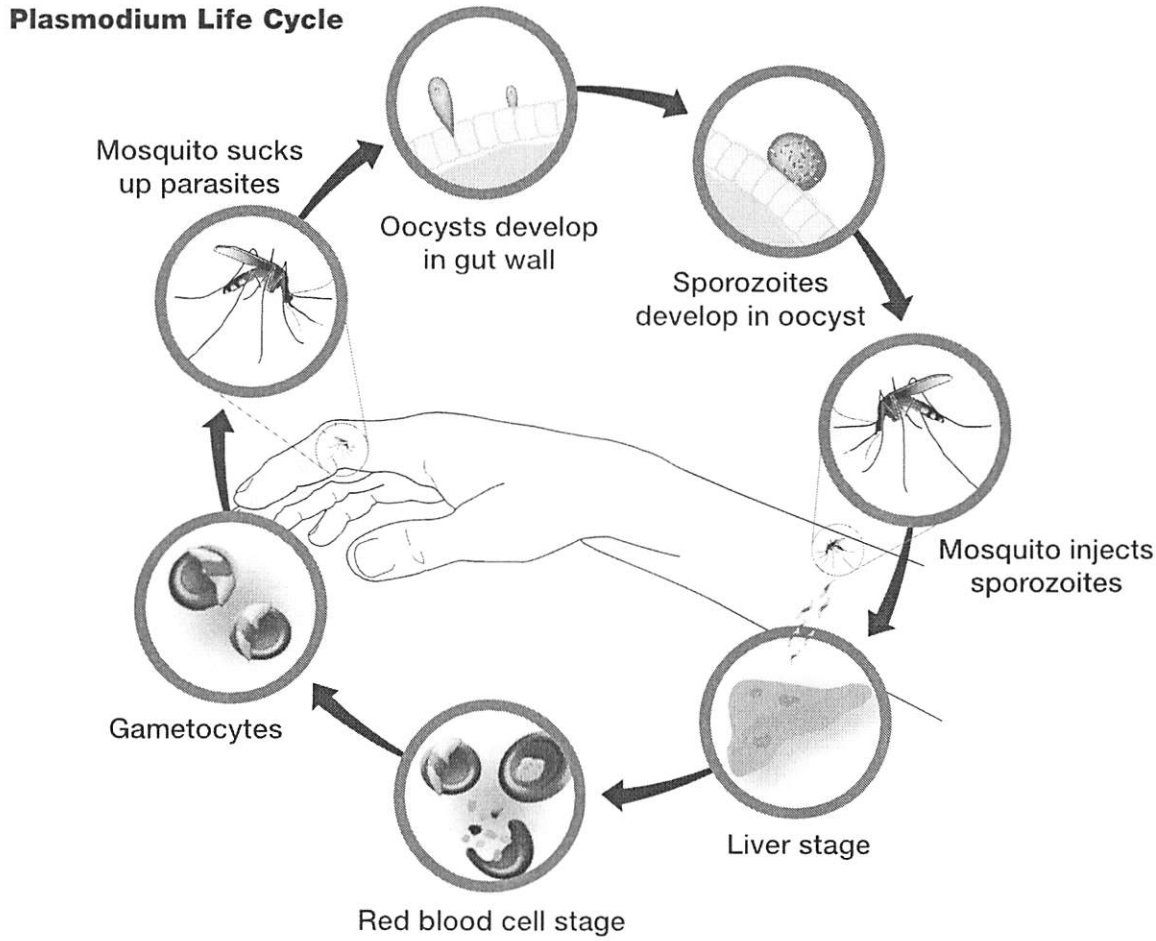
In humans, the *Plasmodium* parasite invades the cells of the liver, lymph nodes, and red blood cells (erythrocytes) in the bloodstream, where it replicates and eventually causes cells to rupture. The human immune system also responds to the invasion, producing the high fevers, nausea, diarrhea, and other flu-like symptoms characteristic of malaria. In mosquitoes, *Plasmodium* lives in the gut and salivary glands; it has no known negative effects on the mosquito host.

As mentioned, *Plasmodium* is mainly transmitted between infected humans by mosquitoes. Specifically, it is spread only by female mosquitoes of the genus *Anopheles*. This is because only female mosquitoes bite humans. They ingest human blood to obtain the proteins necessary for egg development. Most female *Anopheles* mosquitoes are nocturnal feeders (they bite only at night).

When a female mosquito bites and takes blood from a person infected with *Plasmodium*, the microscopic parasite moves along with the human's red blood cells to the mosquito's gut, where it continues through its life cycle. It then moves to the mosquito's salivary gland; when the mosquito takes a bite from another human, *Plasmodium* is injected along with the mosquito's saliva. (It is the proteins in mosquito saliva that trigger an immune response from your body, causing bites to itch.) The parasites can then be ingested by another mosquito, completing the life cycle and transmitting the disease from human to human to human.



Blood smear showing the presence of the* Plasmodium *parasite (crescent shapes). *In the absence of more sophisticated tests, microscopic analysis of blood samples is a common diagnostic approach for malaria in clinics.*



Malaria is passed from person to person through mosquito bites.

Questions

1. Malaria is most often transmitted by mosquitoes, but considering *Plasmodium* lives in erythrocytes, what are other ways in which the disease might spread from human to human?

2. Understanding the biology of malaria, it is not surprising that the most common methods of reducing outbreaks involve mosquito control. Relatively inexpensive and simple to perform, indoor spraying with insecticides kills mosquitoes for 3–6 months, and insecticidal bed nets provide additional protection from bites, with the insecticidal qualities of the nets lasting 3–5 years. What are the main benefits and drawbacks of both these approaches?

Indoor spraying

Benefits:

Drawbacks:

Bed nets

Benefits:

Drawbacks:

3. Malaria is only one of many parasite-mediated, mosquito-borne illnesses affecting the world's population. For this reason, some researchers advocate mosquito elimination — completely killing off mosquito populations in affected regions. The benefits of elimination are obvious (many of us would love to see mosquito-free lakes, streams, and ponds). However, what might some of the concerns be in eliminating a species from a habitat? Do we know what roles mosquitoes play in the environment? Does it make a difference if we know or don't know?

4. Many regions in Africa are reporting increasing populations of *Anopheles* mosquito species that show resistance to insecticides. Even more troubling are findings that climate change is expanding the habitat of the mosquitoes into regions where malaria had not been a health concern. How does your answer to the previous question change if the mosquito in question is an invasive species (for example, in the spread of dengue fever, the mosquito vector in question is often an invasive species new to habitats in North America)?

III. A Novel Approach Involves Bacterial Transformation

Biotechnology and genetic engineering methods are also being investigated as mechanisms for eliminating malaria. Theoretically, any of the species involved — the *Plasmodium* parasite or the human or mosquito host — can be the target of genetic modifications that disrupt either the life cycle or transmission of the parasite. Practically, however, these systems have their drawbacks in terms of their ability to be either cultured or manipulated genetically. Therefore, researchers have turned their attention to a more familiar subject: bacteria.

How can DNA "transform" bacteria? A background to bacterial transformation

In the 1920s, scientists demonstrated how to turn a harmless strain of bacteria into a virulent strain, just by mixing the two strains together (Griffith 1928). What is truly incredible about this experiment is that the virulent strain had been killed prior to mixing, so something in the dead bacteria could "transform" the harmless bacteria, making them virulent.

It wasn't until the 1940s that scientists understood the chemical basis for this transformation. A team of scientists led by Oswald Avery at the Rockefeller Institute found that an extract of the bacteria was unaffected by treatment with protein-digesting enzymes, but was destroyed by a DNA-digesting enzyme. This showed that the agent that transformed the harmless bacteria was DNA (Avery et al. 1944).

Today, we understand that genes within DNA encode proteins that give rise to certain traits. We also know how to exploit the fact that many bacteria can acquire new genes by taking up DNA molecules encoding those genes (for instance, a plasmid) from their surroundings. The process is optimized by adding salts to the transformation medium and using a heat shock step, steps we use deliberately to transform bacteria and other microorganisms. The ability to transform the bacterium *E. coli*, for example, has made possible the cloning of genes, the cornerstone of many modern advances in sciences and of the biotechnology industry.

So how does bacterial transformation relate to our battle against malaria?

Mosquitoes also have gut microbiota?

It is surprising to many that, like humans, mosquitoes harbor a number of symbiotic bacteria within their gut. These symbiotic bacteria can be engineered, using procedures like those you used to transform *E. coli* bacteria, to produce proteins. However, in this case the symbiotic bacteria can be engineered to produce and secrete proteins that interfere with the life cycle of the *Plasmodium* parasite.

In one experiment (Wang et al. 2012), researchers used a bacterium called *Pantoea agglomerans*, which grows abundantly inside *Anopheles* mosquitoes. *P. agglomerans* can be grown and transformed using the same culturing and transformation techniques used with other more common bacteria, like *E. coli*. Researchers used these techniques to engineer *P. agglomerans* to express the genes of the hemolysin (hly) A system of *E. coli* bacteria, three proteins that cause red blood cells to lyse. The transformed bacteria were fed to mosquitoes through sugar solutions. The idea was that when the transformed bacteria colonized the mosquito gut, they would produce the toxic proteins. If that host mosquito then fed upon a human infected with malaria, the toxins produced by the transformed bacteria would cause the red blood cells (from the human blood) to burst. This would halt the life cycle of the *Plasmodium* parasite and stop the spread of malaria.

However, the researchers also hypothesized that transformation and expression of foreign genes might affect the ability of the transformed *P. agglomerans* bacteria to grow or colonize the mosquito gut (in other words, their fitness for that environment might be reduced). This could jeopardize the effectiveness of this strategy for fighting malaria in the wild. So they carried out another experiment: they transformed the bacteria with a plasmid that contains a green fluorescent protein (GFP), derived from the jellyfish *Aequorea victoria*, and fed the transformed bacteria to mosquitoes. The researchers then monitored how much fluorescence came from the mosquito gut. They found that after the host mosquitoes were given a blood meal, the GFP fluorescence in their guts increased, indicating the number of transformed bacteria there had rapidly increased. This demonstrated that transformed *P. agglomerans* could grow in the mosquito gut and, more importantly, replicate quickly when the mosquito ingested a blood meal. This meant the bacteria would also likely produce more hly A proteins when the host mosquito ingested potentially infected blood cells. In terms of the efficacy of the transformed bacteria against the *Plasmodium* parasite, when mosquitoes with the transformed bacteria were fed a blood meal containing the *Plasmodium* parasite, the development of the parasite was inhibited by nearly 98% (Wang et al. 2012).

Questions

1. Scientists have also demonstrated that it is possible to genetically modify the *Anopheles* mosquito to produce a substance in their gut that kills off *Plasmodium*. Why might symbiotic bacteria be a more suitable subject for transformation than the *Anopheles* mosquito?

2. As in the human gut, many different bacterial species inhabit the *Anopheles* gut. If you were the researcher, how would you pick the best species for use in this transformation experiment? What factors should you consider?

3. Why was GFP used in the bacterial transformation experiment?

4. *E. coli* was also used in this experiment for plasmid production (to grow more copies of the plasmids), and the plasmid also contained genes for antibiotic resistance. After transformation, the bacteria were grown on plates with antibiotic in them. Why do you think this is a common step in bacterial transformation?

5. The experiment demonstrated that in the lab the transformed bacteria could survive and proliferate within the mosquito gut after transformation and that they could inhibit *Plasmodium* growth and development by nearly 98%. What other experiments might be needed to demonstrate this is a viable option for malaria elimination in the wild? Consider the life cycles and roles of all the key players in the spread of disease.

6. The hly A system used in the experiment described causes lysis of red blood cells. Considering this, would you have concerns about releasing these transformed bacteria into the environment? Under what conditions might these concerns be alleviated?

7. As a final thought, what do you think the greatest hurdles will be to successfully implement this bacteria-based approach in the wild? What are the technical, ethical, or regulatory challenges, and how might they be handled?

IV: Prognosis

Baruti opens his eyes and sees his mother sitting at his side. She takes his hand and tells him she loves him. She has waited for this moment for three days. She is exhausted from the sleepless nights and constant worry.

The doctors come into the room to check on his progress. They are cautiously optimistic that the mixture of antimalarial drugs is working, and they tell Lerato to be patient. They tell her that her boy was very ill and that treatment takes time, but they are encouraged by the progress he is making. They expect him to recover.

Questions

1. Most antimalarial drugs target the red blood cell (erythrocytic) stage of malaria infection, which is the phase of infection that causes symptomatic illness. Why might it be important to research other medications targeting other stages (for example, the liver stage) of the life cycle? Refer to the figure describing the life cycle of *Plasmodium*.

2. When *Plasmodium* becomes resistant to antimalarial drugs, this results in a delayed or incomplete clearance of the parasite from the patient's blood. How might an organism develop resistance to a chemical that can otherwise kill it?

3. The problem of antimalarial drug resistance can be compounded by cross-resistance, in which resistance to one drug confers resistance to other drugs that belong to the same chemical family or have similar modes of action. How and why do you think this might happen?

4. Current practice in treating cases of malaria is based on combination therapy, in which several different classes of drugs are combined. What might some advantages of this approach be?

5. Many people take antibiotics to treat bacterially mediated illnesses like strep throat or sinus infections. When you take antibiotics, you are told you must take the entire course of the medication in order to reduce the risk of developing antibiotic resistance. Why is a full course needed?