Use of DDT and Alternative Methods to Fight Malaria in Sub-Saharan Africa

by

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Malaria is a disease that seems foreign to many, a distant memory. The World Health Organization reported 216 million cases of malaria across the world in 2016, 445,000 of which resulted in fatalities. While malaria was eliminated in the US in 1951, it's present across the globe, with the epicenter of the reported cases in Sub-Saharan Africa. Malaria is a vector-borne disease, meaning an organism transfers the disease to a host. A vector for malaria is *Anopheles gambiae*, which infects the host with the parasite *Plasmodium.* Elimination has been successful through the use of dichloro-diphenyl-trichloro-ethane (DDT) by spraying the interior of homes. The organic insecticide has been banned globally, but there are still ways to apply for its use. This research focuses on what is used today to fight malaria in Sub-Saharan Africa and methods currently being developed in laboratories. Specifically, a synthesis of secondary materials was conducted looking at the effects of DDT on the environment and human health, mechanisms of *A. gambiae* mutations that lead to insecticide resistance, alternative methods of fighting malaria, and research into new methods of control.

Acknowledgements

I would like to thank Dr. Melissa Graboyes for guiding me through the process of writing my thesis and being so willing to work with me this past year! I am very grateful for Dr. William Bradshaw and Dr. Christina Holzapfel for their willingness to be my advisors from the biology department and taking the time to work with me! It has truly been an honor to work with this team of advisors and I could not have done this without them! Finally, I would like to thank my friends and family who have listened to me talk about my thesis for months, encouraged me when I was stressed about deadlines, and who let me bounce ideas off of them! I felt very supported through this whole process and I'm so grateful for that!

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Abbreviations

ACT: Artemisinin-Based Combination Therapy

CPS: Circumsporozoite protein

DDE: Dichloro-diphenyl-dichloroethylene

DDT: Dichloro-diphenyl-trichloro-ethane

EPA: Environmental Protection Agency

IRS: Indoor Residual Spraying

ITNs: Insecticide-Treated Mosquito Nets

kdr: Knockdown Resistance Mutations

kdr-e: Mutation from Leucine to Serine, originally found in Eastern Africa

kdr-w: Mutation from Leucine to Phenylalanine, originally found in Western Africa

M-form: Inversion of kdr-allele on second chromosome found in Mopti

NANP: N-acetylneuraminic acid phosphatase

NGOs: Non-Governmental Organizations

PCR: Polymerase Chain Reaction

PPF: Pyriproxyfen

S-form: Inversion of kdr-allele on second chromosome, originally found in Savannah

SIT: Sterile Insect Technique

WHO: World Health Organization

Introduction

In 2016, the WHO reported 216 million cases of malaria, 445,000 of which resulted in fatalities. Of the 216 million reported cases in 2016, 90% of the cases and deaths were in Sub-Saharan Africa. Malaria is a vector-borne disease, meaning that it is not transmitted human to human, rather it has another organism that transports the parasite to a new host. The most common vector in Sub-Saharan Africa is *Anopheles gambiae*. Figure 1 show the location of known malaria vectors across the globe. *A. gambiae* is concentrated in Sub-Saharan Africa, but this figure shows how easy it is for malaria to spread to places it may not already be present. Only females infect humans with *Plasmodium*, the parasite that causes malaria, as only females need blood meals to continue the growth of their eggs. Four species of *Plasmodium* can cause malaria: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. *P. falciparum* is the most prevalent and deadly in Sub-Saharan Africa, thus will be species focused on.

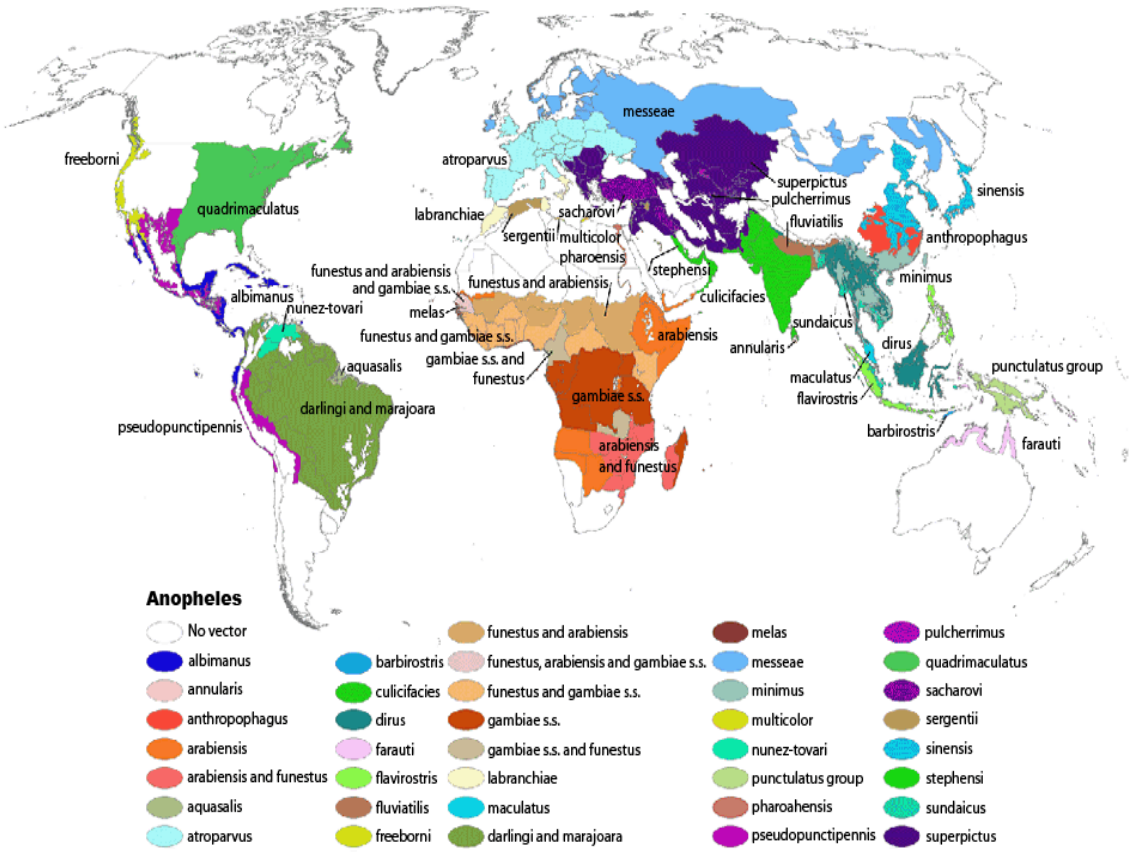

Figure 1: Dispersal of *Anopheles* vectors[1]

Dichloro-diphenyl-trichloro-ethane (DDT) is a well-known insecticide, used extensively across the world until the publication of Rachel Carson's *Silent Spring* brought attention to the environmental effects the insecticide had and led to the formation of the EPA (Environmental Protection Agency) in the U.S.[2] The two main uses of DDT prior to its ban were in agriculture to ward off pests and to fight malaria. Countries such as the United States and Italy had used DDT to fight malaria and

[1] A Kiszewksi et al. "A Global Index Representing the Stability of Malaria Transmission." *American Journal of Tropical Medicine and Hygiene* 70, no. 5 (2004): 486-498. https://www.ncbi.nlm.nih.gov/pubmed/15155980.

[2] S Sadasivaih, Y Tozan, and JG Breman. "Dichlorodiphenyltrichloroethane (DDT) for Indoor Residual Spraying in Africa: How Can It Be Used for Malaria Control?" *The American Journal of Tropical Medicine and Hygiene* 77, no. 6 (2007): 249-263. https://www.ncbi.nlm.nih.gov/books/NBK1724/.

succeeded in its elimination in those areas.[3] IRS is done by spraying the interior walls of houses with an insecticide. Once the mosquito takes a blood meal, it need to rest in order to metabolize the blood. This resting period can take place on walls, depending on the species of mosquito, thus when they land on a wall that has been sprayed with the insecticide, the mosquito absorbs the chemical through their feet, resulting in death. This method was successful yet was put to a stop after the ban of DDT by the US in 1972 and its use restricted globally at the 2001 Stockholm Convention on Persistent Organic Pollutants.[4] In insects, DDT affects the sodium potassium channel in neurons, preventing concurrent action potentials from stopping.[5] Once the walls have been sprayed mosquitoes will absorb the insecticide when they land, thus resulting in their death.[6] Due to the effectiveness of DDT and its slow rate of decay, the walls of individual homes only have to be sprayed every 6 months.[7]

Today, malaria is still a problem across the world, specifically Sub-Saharan Africa. Partially due to an increase in population and travel, exposure to malaria has likely increased.[8] Malaria, when undetected, can cause death and unfortunately, death is unequally proportional in children, with 70% of the malaria deaths overall in children under the age of 5.[9] The symptoms of malaria include a severe headache, fever cycles,

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[3] Ibid.
[4] S Sadasivaih, Y Tozan, and JG Breman. "Dichlorodiphenyltrichloroethane (DDT) for Indoor Residual Spraying in Africa: How Can It Be Used for Malaria Control?" *The American Journal of Tropical Medicine and Hygiene* 77, no. 6 (2007): 249-263. https://www.ncbi.nlm.nih.gov/books/NBK1724/.
[5] Ibid.
[6] Randall Packard. *A History of Global Health: Interventions into the Lives of Other Peoples*. Baltimore: Johns Hopkins University Press, 2016. 107.
[7] Ibid.
[8] James Webb. *The Long Struggle Against Malaria in Tropical Africa*. New York, New York: Cambridge University Press, 2014. 190.
[9] T Tizifa et al. "Prevention Efforts for Malaria." *Current Tropical Medicine Reports* 5, no.1 (2018): 41-50. doi: 10.1007/s40475-018-0133-y.

nausea, and chills. Often adults are asymptomatic, or the symptoms are quite similar to other diseases common in their area, making diagnosis even more difficult.[10]

Acquired immunity is common in adults in areas where malaria is often present. Children may be repeatedly infected with malaria, but after each infection their immune system becomes stronger. If they survive into adulthood, each case of malaria may become less severe and the likelihood of death decreases. The time in which this acquired immunity takes to form is unknown as are many of the specifics of the process. If one with acquired immunity leaves an area with a high occurrence of malaria and returns a while later, immunity may be lost. Immunity can also be lost if malaria is eliminated from an area and there is no longer exposure to the disease consistently. This can lead to rebound malaria.

Rebound malaria occurs when acquired immunity is lost and then the disease is reintroduced. Malaria is then more difficult to fight off and can have high mortality rates in those of all ages. This can be caused when a malaria campaign is not entirely successful or an infected person travels to the area and is bitten by a mosquito, restarting the cycle. A lack of funding, thus halting control methods could end in rebound malaria as well. There has been a significant drop in IRS funding since 2010, which is not ideal for control but also rebound malaria.[11] Choosing the right method of control for each specific area and making sure it can be sustained to prevent rebound malaria is vital.

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[10] Melissa Graboyes. *The Experiment Must Continue: Medical Research and Ethics in East Africa, 1940-2014*. Athens, Ohio: Ohio University Press, 2015. 170-171.

[11] Melissa Graboyes. *The Experiment Must Continue: Medical Research and Ethics in East Africa, 1940-2014*. Athens, Ohio: Ohio University Press, 2015. 170-171. (Source for both paragraphs).

Attempts to control malaria today vary greatly, from IRS with pyrethroids to research into sterility and vaccines. There is no cohesive solution to the problem of malaria. A problem this complex takes time and lots of funding, which is lacking. This thesis looks at current methods of control, research into new techniques, and the mechanisms behind resistance will allow for a better understanding of why elimination has not been reached in Sub-Saharan Africa.

Importance of Control

Control of malaria is not as easy as it was back when malaria was eliminated in the United States. The WHO suggests that the main reasons why elimination has not occurred yet are: a lack of funding, conflicts that prevent NGOs from entering, the vastly changing climate due to global warming, and the resistance in both mosquitoes and parasites. One roadblock to detection is that the importance of fevers and their needing medical attention differs culturally.[12] Rural areas have a harder time accessing quick diagnostic technology or effective treatment if given a positive diagnosis for malaria.[13] Another issue is that *A. gambiae* is most active in the early evening, thus humans are moving about and making it quite possible that *A. gambiae* never comes into contact with an insecticide, either in nets or on walls. Alternative methods that can account for the movement of both humans and mosquitos is vital for successful control.

The increase in global temperature is linked to malaria in a multitude of ways. The blood meal leading to egg development, hatching of the egg, and survival of the mosquitos are all temperature-dependent.[14] *A. gambiae* prefers a specific habitat, one usually produced after deforestation or construction, that includes small pools of warm water, containing algae for the larva, and have little to no predators nearby.[15] Simply put, *A. gambiae* can spread *Plasmodium* much more effectively as the effects of global

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[12] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1

[13] Ibid. 28.

[14] Eikenberry SE and AB Gumel. "Mathematical modeling of climate change and malaria transmission dynamics: a historical review." *Journal of Mathematical Biology* (2018): 1-77. doi: 10.1007/s00285-018-1229-7.

[15] Ibid.

warming increase as the increase in temperature works to their advantage.[16] Thus, global warning increases both infection of malaria and its geographical distribution.[17] Increased rainfall and humidity, associated with climate change, will allow for *A. gambiae* to thrive and breed in high altitudes they normally could not and will possibly have a faster lifecycle in areas where malaria is already present.[18] Droughts can cause shallow rivers, thus creating more habitat for the mosquitos.[19] There is the possibility that too much rain can wipeout malaria breeding grounds, but not enough research has been conducted to verify that this has an effect on transmission rates.[20]

[16] Ibid.

[17] L Ivanescu et al. "Climate Change Is Increasing the Risk of the Reemergence of Malaria in Romania." *Biomedical Research International* (2016). doi: 10.1155/2016/8560519.

[18] Craig M.H, RW Snow, and D le Sueur. "A Climate-based Distribution Model of Malaria Transmission in Sub-Saharan Africa." *Parasitology Today* 15, no. 3 (1999): 105-111. doi: 10.1016/S0169-4758(99)01396-4.

[19] Ibid.

[20] Ibid.

Current Methods of Malaria Control

Two methods of control used in Sub-Saharan Africa are IRS and bed nets. From 2014 to 2016, 505 million insecticide-treated mosquito nets (ITNs) were distributed in Sub-Saharan Africa, 75% of which were distributed through specific distribution campaigns. All ITNs currently used in Sub-Saharan Africa are treated with pyrethroids. 80% of people in areas with a high occurrence of malaria cases have at least 1 ITN in their home, but it may not be enough. 45 million people in Sub-Saharan Africa had IRS conducted in their homes in 2016, but this number is much lower than previous years due to resistance to specific insecticides, causing the need of more expensive chemicals to replace them. Pyrethroids are the main insecticide used for IRS, but others have become more abundant, as seen in Figure 2.[21] From 2010 to 2012 there was an increasing trend of pyrethroid only use and then a steady decline from 2013 to 2016. The use of other insecticides has increased from 2010 to 2016, suggesting that resistance to pyrethroids has resulted in the need to use other insecticides for vector control.

[21] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1 (Source for entire paragraph).

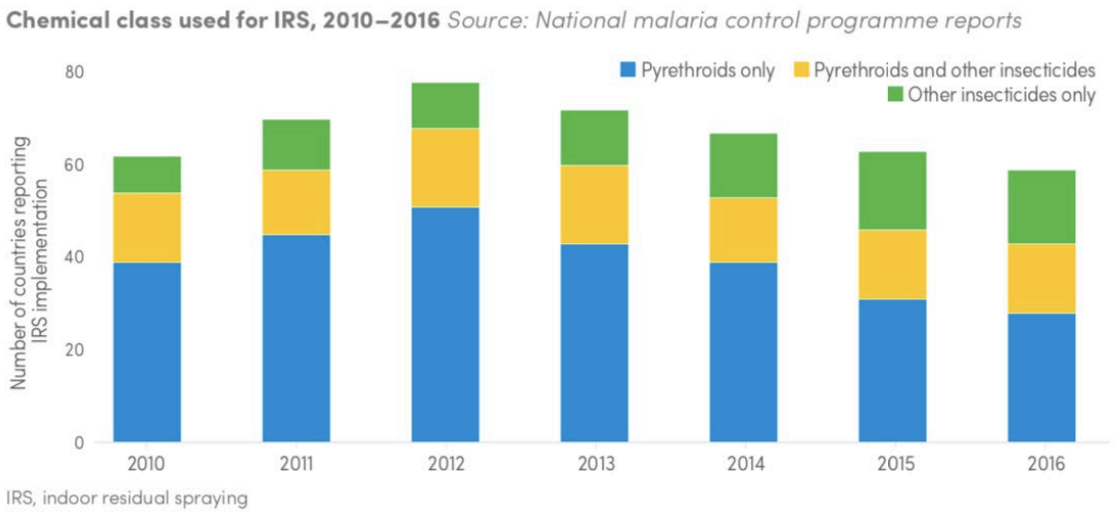

Figure 2: Insecticide use over time[22]

Vector control is not limited to IRS and mosquito nets. Larval source management is used where larvicides that pre-approved by the WHO are sprayed into water sources, killing the growing larva.[23] Known habitats will be removed, and often landscape is changed to prevent pooling of shallow water. Natural enemies, such as fish, can be added to the habitats as well. Effort has been put into improving homes to minimize ways in which mosquitos can enter, such as putting screens on doors and windows and making sure homes have ceilings. Attractive toxic sugar bait is also used, where overly ripe local fruit or flowers are added to a pool of sugar, mixed with a toxin (spinosad or boric acid usually) and sprayed on outside vegetation or put in a standard area. This method is inexpensive, but not much is known about its effectiveness.[24]

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[22] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1

[23] "Controlling Mosquitoes at the Larval Stage." United States Environmental Protection Agency. https://www.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage.

[24] T Tizifa et al. "Prevention Efforts for Malaria." *Current Tropical Medicine Reports* 5, no.1 (2018): 41-50. doi: 10.1007/s40475-018-0133-y. (Source for entire paragraph).

Different campaigns involving chemoprevention have begun throughout Sub-Saharan Africa. For women who are pregnant, the WHO recommends preventative therapy with sulfadoxine-pyrimethamine, an antimalarial drug. 19% of pregnant women in the 23 countries that reportedly used this policy received the suggested three doses in 2016. The seasonal malaria chemoprevention program was implemented in 12 countries, allowing for 15 million children to be given ACT (artemisinin-based combination therapy) in areas where transmission is very high during certain times of the year. The integrated community case management program was also enacted by the WHO, which trains community workers to look for other diseases, including malaria. Malaria surveillance systems have also been put in place, which in theory allow for cases and deaths due to malaria to be reported and allow for appropriate resources to be allocated.[25]

While these statistics on control efforts have been reported by the WHO, it needs to be taken into consideration that there is little consistency in reporting and confirming malaria cases with the correct diagnostic techniques. Rapid diagnostic tests have been distributed, but still require a blood test and are not as accurate as microscopy in diagnosing malaria. It has also become increasingly difficult to identify if one has malaria as a histidine-rich protein 2 gene deletion in *P. falciparum* has effected the efficacy of malaria diagnostics. The parasite with the deletion will cause a false negative result in a rapid diagnostic test, which can cause major issues with detection

[25] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1 (Source for entire paragraph).

and accurate reporting of the cases of malaria. The WHO has estimated the amount of malarial cases by looking at the prevalence of *P. falciparum* and the geographical location of the cases that were reported, but the accurate number is unknown.

Insecticides

DDT consist of two chlorobenzenes and a trichloromethyl radical connecting the two.[26] It is not soluble in water, but since it is an organic compound it is quite soluble in organic solvents.[27] DDT is easily oxidized through the trichloromethyl radical, thus how it can be metabolized.[28] The high solubility of DDT in organic solvents allows it to easily enter the environment.[29] The primary metabolite is DDE, which has a half-life of 11 years, meaning that once it is enters the environment it will be there for a long time.[30] DDT very slowly breaks down in the soil, but the process is much faster in tropical areas as microorganisms degrade it at a faster pace.[31] Since DDT is not soluble in water, once it reaches surface water it will "settle and be deposited in the sediment".[32] This allows fish and other marine life to absorb DDT.[33]

While the presence of DDT and DDE metabolites in the environment is undeniable, the effects of its presence are debatable. The thinning of eggs shells and effects on bird reproduction are common topics of study in terms of the known effects of DDT. This is due to bioaccumulation in the ecosystem and has dramatically effected population sizes. Humans can be exposed to DDT through food consumption, inhalation, and absorption through the skin. Once in the body, DDT is broken down

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[26] Ben Lim. *DDT: Chemistry, Metabolism, and Toxicity*. Washington D.C: Environmental Protection Agency, 1972. 1.
[27] Ibid.
[28] Ibid.
[29] Ben Lim. *DDT: Chemistry, Metabolism, and Toxicity*. Washington D.C: Environmental Protection Agency, 1972. 1.
[30] S Sadasivaih, Y Tozan, and JG Breman. "Dichlorodiphenyltrichloroethane (DDT) for Indoor Residual Spraying in Africa: How Can It Be Used for Malaria Control?" *The American Journal of Tropical Medicine and Hygiene* 77, no. 6 (2007): 249-263. https://www.ncbi.nlm.nih.gov/books/NBK1724/.
[31] "Public Statement of Health." Agency for Toxic Substances & Disease Registry. 2002, https://www.atsdr.cdc.gov/phs/phs.asp?id=79&tid=20.
[32] Ibid.
[33] Ibid.

into metabolites, which are stored in fatty tissue. The only way it can leave the body is through urine and occasionally in breast milk. Consuming large amounts of DDT could affect the nervous system, but "no effects have been reported in adults given small daily doses of DDT." Since overall DDT levels have dramatically decreased since the ban of DDT, the overall human effects are limited.[34] According to a review published in the *American Journal of Tropical Medicine and Hygiene*, "it has been suggested that because of its weak estrogenic activity, DDT exposure is linked to great cancer; [but] there is no strong evidence to support this association."[35] A link to cancer could be a major factor in the decision to use DDT. The unknown effects are difficult to argue as while there is no concrete data that DDT is harmful, scientists don't know if it's safe either. Much more research needs to be conducted on how DDT enters human tissues and the possibly harmful effects of its presence to fully understand its effects on human health.

Despite the massive amounts of research dedicated to DDT and its effects on the environment, little is known about what it truly does in the human body. The WHO's current stance is that "DDT does not pose a major risk to human health when used within the approved guidelines."[36] Their stance is very vague, only claiming that there isn't a major effect. By stating that DDT is safe when used the way they suggest allows them to evade any blame if something does happen and human health is effected in

[34] "Public Statement of Health." Agency for Toxic Substances & Disease Registry. 2002, https://www.atsdr.cdc.gov/phs/phs.asp?id=79&tid=20. (Source for entire paragraph).
[35] S Sadasivaih, Y Tozan, and JG Breman. "Dichlorodiphenyltrichloroethane (DDT) for Indoor Residual Spraying in Africa: How Can It Be Used for Malaria Control?" *The American Journal of Tropical Medicine and Hygiene* 77, no. 6 (2007): 249-263. https://www.ncbi.nlm.nih.gov/books/NBK1724/.
[36] James Webb. *The Long Struggle Against Malaria in Tropical Africa*. New York, New York: Cambridge University Press, 2014. 186.

some way. The idea that DDT may cause cancer is a concern but could be used as scare tactic to curb favor against the use of DDT. Science has come a long way since DDT was first synthesized, but our general understanding has not. Another factor in this debate is that much less DDT would be used in IRS than in its height of usage. Most of the DDT was used in mass amounts for agricultural purposes and only a fraction of the total usage was for IRS.[37] Even if that same amount was used for malaria control in the future, the environmental effects would be much lower than during the original campaign. The levels should never go back to the way they were prior to its restriction, its use should still be restricted to vector-borne disease control. DDT is currently being used for IRS, but only in certain cases where correct paperwork has been done and approval has been given.

Overall, there are three classes of insecticides used today for IRS: carbamates, organophosphates, and pyrethroids.[38] Carbamates are not as prevalent today and the WHO recommends mostly pyrethroids and pyrethrins for IRS and treating mosquito nets.[39] Pyrethrins are insecticides derived from chrysanthemum flowers and pyrethroids are synthetic versions of pyrethrins.[40] They have a low cost, low toxicity to humans, and so effective that they are the most used insecticide in malaria control across the globe.[41]

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[37] S Sadasivaih, Y Tozan, and JG Breman. "Dichlorodiphenyltrichloroethane (DDT) for Indoor Residual Spraying in Africa: How Can It Be Used for Malaria Control?" *The American Journal of Tropical Medicine and Hygiene* 77, no. 6 (2007): 249-263. https://www.ncbi.nlm.nih.gov/books/NBK1724/.
[38] K Walker. "Cost-comparison of DDT and alternative insecticides for malaria control." *Medical and Veterinary Entomology* 14, no. 4 (2008), 345-354. doi: 10.1046/j.1365-2915.2000.00262.x.
[39] Ibid.
[40] "Pyrethrins and Pyrethroids." United States Environmental Protection Agency. https://www.epa.gov/ingredients-used-pesticide-products/pyrethrins-and-pyrethroids.
[41] C Ngufor, A Fongnikin, M Rowland, and R N'Guessan. "Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant *Anopheles gambiae* sl in Southern Benin." *PLoS One* 12, no. 12 (2017). doi: 10.1371/journal.pone.0189575.

They are fast acting and follow similar pathways of DDT in terms of mosquito mortality, but they do not bioaccumulate as much as DDT does.[42] These insecticides, since they were used in both nets and IRS throughout Sub-Saharan Africa, have become quite resistant to mosquitos, thus their efficacy has greatly decreased over time.[43]

Studies have been conducted looking at the effect of mixing pyrethroids with other insecticides that are not related mechanistically to be used in areas where resistance is present. One in particular looked at the use of clothianidin and deltamethrin. Clothianidin is a neonicotinoid, where is an agonist for nicotinic acetylcholine receptors in neurons. It was originally used in agriculture to kill broad spectrum pests and by veterinarians to prevent fleas in cats and dogs. This means that the insecticide kills mosquitoes differently than DDT and pyrethroids, thus less resistance may be possible. It causes acute toxicity by contact and stomach poisoning, which can take longer than pyrethroids and may delay death. By mixing together clothianidin and deltamethrin (a pyrethroid), the mortality of mosquitos increased in comparison to the two insecticides alone. More research is currently being done on Fludora, a solution of these two insecticides currently in Phase III clinical trials.[44] By looking into mixing insecticides in IRS, there is a chance for less resistance and less of a need to rotate chemicals.

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[42] M Moreno et al. "Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea." *Tropical Medicine & International Health* 13, no. 3(2008):430-3. doi: 10.1111/j.1365-3156.2008.02010.

[43] Ibid.

[44] C Ngufor, A Fongnikin, M Rowland, and R N'Guessan. "Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant *Anopheles gambiae* sl in Southern Benin." *PLoS One* 12, no. 12 (2017). doi: 10.1371/journal.pone.0189575. (Source for entire paragraph).

The cost of fighting malaria is important to keep in mind. Even though the cost of IRS with DDT is the least expensive of the options for control, many countries are either unable or unwilling to cover the cost. The WHO estimates that to meet the 2030 targets of getting closer to elimination, it will cost $6.5 billion annually until at least 2020.[45] This is made even more difficult as since 2014 the donations from high-risk countries have steadily decreased, placing the burden on wealthy, unaffected countries.[46] The United States is the largest funder of malaria control, while the US Government National Institutes of Health, the Bill and Melinda Gates Foundation, and pharmaceutical and biotechnology companies fund most of the research into malaria treatment and future control methods.[47]

The cost of using DDT or other insecticides for IRS varies. Walker conducted a study comparing the price of DDT and other insecticides common in IRS.[48] In 2000, the cost per household of DDT IRS per 6 months was $1-$3 USD. In comparison, Ficam, a common carbamate used in IRS costs $2-$5.50 USD.[49] On the large scale in which IRS is needed, this small difference in cost matters greatly. Another study conducted by Walker in 2008 suggests that DDT is still the cheapest insecticide for IRS, but the price may increase over time as production has greatly decreased due to bans on the insecticide in many countries.[50] Pyrethroids are sprayed at a lower dosage than

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[45] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1

[46] Ibid.

[47] Ibid. xiii, 6.

[48] K Walker. "Cost-comparison of DDT and alternative insecticides for malaria control." *Medical and Veterinary Entomology* 14, no. 4 (2000), 345-354. doi: 10.1046/j.1365-2915.2000.00262.x.

[49] Ibid.

[50] K Walker. "Cost-comparison of DDT and alternative insecticides for malaria control." *Medical and Veterinary Entomology* 14, no. 4 (2008), 345-354. doi: 10.1046/j.1365-2915.2000.00262.x.

DDT, but must be sprayed multiple times a year, depending on the area.[51] While the largest cost associated with IRS is that of the cost of the insecticide used, the cost analysis done by Walker does not take into account safety, transportation and operational costs.[52] These costs will vary greatly in each country, thus it is recommended that each country determine the best insecticide from the WHO approved list that fits their budget.[53] DDT is not currently on the list, despite suggestions of the WHO that DDT is safe to use for IRS.[54]

[51] Ibid.
[52] Ibid.
[53] Ibid.
[54] "Prequalification Vector Control." WHO. http://www.who.int/pq-vector-control/prequalified-lists/en/

Knockdown Resistance Mutations

This ability of *A. gambiae* to eventually resist insecticides directly relates to molecular evolution. In general, the relationship between *Plasmodium* and *A. gambiae* is due to evolution, as the mosquitoes became more susceptible to the parasite over time.[55] The DNA of *A. gambiae* quickly evolves (within 2 years) and these mutations can be tracked through phylogenies. A selective pressure is forcing the mosquitoes to evolve through knockdown resistance mutations (kdr).[56] The kdr mutations have been found in thirteen different *Anopheles* species in Africa, Asia, and the Americas.[57] By tracking where the mutations originated and where they are now, scientists could not only understand how the mutations came about but find ways to avoid resistance to certain insecticides in the future. An example of this is pyrethroids.[58] They were thought to have been a great alternative to DDT that *A. gambiae* would not become resistant to, but recently resistance and knockdown mutations have become prevalent. In 61 of the 76 countries where the WHO operates for malaria control, there is resistance to at least one insecticide and an 81% overall resistance to pyrethroids specifically.[59] It is suggested that the first single point mutation that lead to kdr

[55] M Ousmane Ndiath et al. "Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to *Plasmodium falciparum*: a hindrance for vector control." *Malaria Journal* 13, no. 340 (2014). doi: 10.1186/1475-2875-13-340.
[56] AP Silva, JM Santos, and AJ Martins. "Mutations in the voltage-gated sodium channel gene or anophelines and their association with resistance to pyrethroids- a review." *Parasites & Vectors* 7, (2014): 450. doi: 10.1186/1756-3305-7-450.
[57] Ibid.
[58] M Moreno et al. "Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea." *Tropical Medicine & International Health* 13, no. 3(2008):430-3. doi: 10.1111/j.1365-3156.2008.02010.
[59] "World malaria report 2017." World Health Organization, Geneva, 2017. License: CC BY-NC-SA 3.0 IGO. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=F811BC0ACA6BC258DAE196E8A185ED9D?sequence=1

occurred due to the intense use of DDT prior to its ban and the use of pyrethroids for crops, thus the cross-resistance.[60]

There are two mechanisms for mutation in *A. gambiae*, knockdown resistance and metabolic resistance. Metabolic resistance involves the mosquito using detoxification enzymes (cytochrome P450 and glutathione-S-transferase) to breakdown the insecticide before it reaches the sodium channels in the brain's nervous system, which is how the insecticide kills the mosquito.[61] Mutations in coding genes for these enzymes allow for too much of the enzyme to be made, thus the faster metabolism of the insecticide.[62] While the biochemistry of metabolic resistance is complex and less understood, much more is known about kdr mutations. Knockdown resistance mutations are target-site mutations within the *para* sodium channel gene that prevent the insecticide from binding.[63]

Knockdown refers to the paralysis and spasms that the insects have when they come into contact with a lethal dose of an insecticide.[64] Both pyrethroids and DDT target the sodium channels, but the kdr mutations are conserved between species, thus resistance is widespread. This suggests that convergent evolution is occurring. *A. gambiae* had non-synonymous mutations occur independently multiple times in the L1014 position, but some took one or two generations to come to fruition.

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[60] M Ousmane Ndiath et al. "Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to *Plasmodium falciparum*: a hindrance for vector control." *Malaria Journal* 13, no. 340 (2014). doi: 10.1186/1475-2875-13-340.
[61] X Chang et al. "Landscape genetic structure and evolutionary genetics of insecticide resistance gene mutations in *Anopheles sinensis*." *Parasites & Vectors* 23, no. 9 (2016): 228. doi: 10.1186/s13071-016-1513-6.
[62] AP Silva, JM Santos, and AJ Martins. "Mutations in the voltage-gated sodium channel gene or anophelines and their association with resistance to pyrethroids- a review." *Parasites & Vectors* 7, (2014): 450. doi: 10.1186/1756-3305-7-450.
[63] Ibid.
[64] Ibid.

Geographical landscape has a large effect on the kdr mutations as gene flow is limited between populations. For example, a study was conducted in China using PCR to look at kdr mutations at the L1014 position. Mosquitoes with kdr mutations in central China had reached fixation, but those is southern China had few kdr mutations present.[65] They also suggested that genetic hitchhiking played a role in the increase of kdr mutations in central China, thus limited variation and fixation of the mutations.[66]

There are two specific substitutions that results in the kdr mutation: leucine replaced by phenylalanine, first found in West Africa (kdr-w), and leucine replaced by a serine, originally found in East Africa (kdr-e).[67] The first lineages of kdr were found in Africa, thus the specific names for their specific alleles.[68] Equatorial Guinea and Uganda have found both kdr-w and kdr-e, thus many *A. gambiae* in those areas are heterozygotes for the mutations.[69] There are 5 different molecular forms of the kdr mutation due to inversions on the second chromosome, the most common being S and M.[70] The S-form refers to the mutation found in the Savannah form and the M-form refers to the Mopti form.[71] Both forms had originally occurred separately, but due to introgression of the S-form, both can now be found in the same areas.[72] Despite the

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[65] Ibid.
[66] X Chang et al. "Landscape genetic structure and evolutionary genetics of insecticide resistance gene mutations in *Anopheles sinensis*." *Parasites & Vectors* 23, no. 9 (2016): 228. doi: 10.1186/s13071-016-1513-6. (Source for entire paragraph).
[67] M Moreno et al. "Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea." *Tropical Medicine & International Health* 13, no. 3(2008):430-3. doi: 10.1111/j.1365-3156.2008.02010.
[68] AP Silva, JM Santos, and AJ Martins. "Mutations in the voltage-gated sodium channel gene or anophelines and their association with resistance to pyrethroids- a review." *Parasites & Vectors* 7, (2014): 450. doi: 10.1186/1756-3305-7-450.
[69] M Moreno et al. "Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea." *Tropical Medicine & International Health* 13, no. 3(2008):430-3. doi: 10.1111/j.1365-3156.2008.02010.
[70] Ibid.
[71] Ibid.
[72] Ibid.

introgression event, there is still very little gene flow between the S and M-forms due to very few hybrid genotypes.[73] This has been found to be consistent throughout different geographical areas.[74] Kdr-w alleles are found in both forms, but the S-form more frequently.[75] Kdr-e alleles have only been found in the S-form.[76] In a study on the heterozygous *A. gambiae* mutants in Equatorial Guinea, researchers found a decreased mortality rate with DDT, but not pyrethroids.[77] While no direct conclusions could be made about heterozygous mutations, this study brought up the importance of heterozygosity in target-site mutations and how little is known about them.[78]

There is still some ambiguity as to if the kdr mutations directly correlate to resistance. To test this, researchers usually conduct a bioassay with the insecticides and use PCR to genotype the kdr mutation in both the dead and surviving mosquitoes. In a meta-analysis of ninety-eight studies that tested this correlation, sixty-three saw a correlation between kdr mutations and resistance. Six of them suggested that both kdr mutations and metabolic resistance lead to the mosquitos' resistance. Six other studies did not find a link but suggested that may be due to a small sample size. The last twenty-eight studies did not conduct a bioassay but found the kdr mutations after conducting PCR and concluded that just having the mutation meant resistance would occur. Regardless, it is recommended locals attempt to reduce the selective pressure on

[73] M Ousmane Ndiath et al. "Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to *Plasmodium falciparum*: a hindrance for vector control." *Malaria Journal* 13, no. 340 (2014). doi: 10.1186/1475-2875-13-340.
[74] Ibid.
[75] M Moreno et al. "Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea." *Tropical Medicine & International Health* 13, no. 3(2008):430-3. doi: 10.1111/j.1365-3156.2008.02010.
[76] Ibid.
[77] Ibid.
[78] Ibid.

A. gambiae to prevent resistance. This can be done by using less insecticides, but enough to still be lethal. Another way to avoid resistance is to periodically switch which insecticide is being used and chose ones that have different mechanisms.[79]

Another study was conducted to determine the effect of kdr mutations and mating of *A. gambiae*. The researchers found that heterozygous males for the kdr mutation were much more likely to mate than males who were homozygous resistant. This suggested that not only do kdr mutations effect mating, but it makes a difference if the mosquito has one or two copies of the kdr mutant. This study also suggested that the mating issue could possibly prevent fixation and slow the rate of evolution of kdr. Also, the homozygous males may not be able to find a female to mate with fast enough and could be outcompeted by the heterozygous males. This is due to the fact that sodium channels are vital to send olfactory cues and since kdr mutations effect sodium channels, the homozygous males may not be able to smell these cues from the females.[80]

While the kdr mutations are most likely correlated to resistance of insecticides, their effect on the susceptibility of *A. gambiae* to *Plasmodium* is vital for the continuation of the disease. A study showed that there is no palpable difference in susceptibility in the S-form, but those who are homozygous mutants are more

[79] AP Silva, JM Santos, and AJ Martins. "Mutations in the voltage-gated sodium channel gene or anophelines and their association with resistance to pyrethroids- a review." *Parasites & Vectors* 7, (2014): 450. doi: 10.1186/1756-3305-7-450. (Source for entire paragraph).
[80] N Platt et al. "Target-site resistance mutations (kdr and RDL), but not metabolic resistance, negatively impact male mating competitiveness in the malaria vector *Anopheles gambiae*." *Heredity* 115, no. 3 (2015): 243-252. doi: 10.1038/hdy.2015.33. (Source for entire paragraph).

susceptible than heterozygotes.[81] Mosquitoes without the kdr mutant were the most susceptible to *Plasmodium* of all the mutants.[82] The researchers noted that metabolic resistance may have even more of an effect on the variations of *Plasmodium* and *A. gambiae's* susceptibility.[83] Since mosquitoes can only be infected once, how fast mosquitoes become infected has to do with their immune system.[84]

[81] M Ousmane Ndiath et al. "Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to *Plasmodium falciparum*: a hindrance for vector control." *Malaria Journal* 13, no. 340 (2014). doi: 10.1186/1475-2875-13-340.
[82] Ibid.
[83] Ibid.
[84] Ibid.

Research into Vector Control

Male sterility has become of great interest in terms of fighting malaria. The sterile insect technique (SIT), where sexually competitive sterile males are released into areas with a large number of reported malaria cases to mate with the females to prevent new generations of mosquitos infected with *P. falciparum*, is an exciting option for future vector control. The males are sterilized through irradiation using gamma or X-rays. While in theory, this method could have a large impact on malaria, there are some issues that need to be worked out first. In order to have an impact on large populations, a mass number of sterile males must be released in order to outnumber the wild males. This requires large quantities of mosquitos reared in laboratories, which can possibly lead to modified behavior, inbreeding, and a loss of genetic diversity. These effects can lead to a reduced competitiveness with wild males, rendering SIT inviable as a control technique, as inbreeding can lead to lower vigor and behavior is an important aspect of mating. Body size is another important factor in mating, but a study conducted in 2017 suggests that mass produced sterile males have the same body size and life span of wild males, allowing them to be competitive.[85] More research needs to be done on how to properly carry out SIT, as well as finding a way to make it economically comparable to that of IRS and mosquito nets.

One method currently being researched concerning genetic mutations is that of female sterility. Most of the current methods of vector control target adult mosquitoes, but female sterility focuses on the production of new mosquitoes to carry the parasite.

[85] D Soma et al. "Does mosquito mass-rearing produce an inferior mosquito?" *Malaria Journal* 16, no.1 (2017): 357. doi: 10.1186/s12936-017-2012-8. (Source for entire paragraph).

Since the females are the only ones who spread *Plasmodium*, by decreasing their ability to reproduce the likelihood of malaria spreading is greatly reduced. Pyriproxyfen (PPF) is a hormone, currently being investigated in field trials, that is used to prevent metamorphosis by effecting the development of female ovaries. PPF is very effective in small doses and has a low toxicity to humans. In theory, this is a great method, but resistance is possible as the mechanisms involved in PPF sterilization involve P450 enzymes. P450 enzymes breakdown the PPF, but mutations in expression of these enzymes were found to be a part of *A. gambiae* resistance to DDT and pyrethroids. It is suggested that a strain of mosquitos that are resistant to pyrethroids have a chance of already being resistant to PPF as the enzymes targeted for resistance can breakdown PPF.[86]

Another way in which genetic mutations can be used to control malaria has to do with biting. By preventing female mosquitoes from biting, malaria will not be transmitted. There are three genera of mosquitos that do not bite and some species that do not bite on their first ovarian cycle but must for future cycles. As nonbiting mosquitos do exist in nature, understanding how they evolved from biting mosquitoes is imperative for their possible use in the future for vector control. Since biting and nonbiting species arose from independent evolution events and are incompatible, it is not feasible to compare gene expression. To understand the evolution of nonbiting, Bradshaw et al looked at a species of mosquito that bites in one geographical area and does not bite in another, *Wyeomyia smithii*. By artificially selecting for biting mosquitos

[86] C Yunta et al. "Pyriproxyfen is metabolized by P450s associated with pyrethroid resistance in *An. gambiae*." *Insect Biochemistry and Molecular Biology* 78, (2016): 50-57. doi: 10.1016/j.ibmb.2016.09.001. (Source for entire paragraph).

and then comparing gene expressing prior to feeding, Bradshaw et al found that nonbiting mosquitoes evolved through natural selection, rather than genetic drift. This is important because it suggests that nonbiting was a favorable trait for certain species in nature and was in no way a random accident. It was also suggested that nonbiting mosquitoes are prepared to allocate resources, like acetyl-CoA, to multiple pathways while biting mosquitos progress through to prepare for the eventual metabolism of a blood meal.[87] This study is vital to the field of evolution and genetics because it demonstrates how a possibly permanent solution to malaria transmission could be found using genetics. By understanding more about the genes involved in nonbiting and using laboratory processes, like CRISPR/Cas9, the way scientists go about preventing disease could change.

[87] WE Bradshaw et al. "Evolutionary transition from blood feeding to obligate nonbiting in a mosquito." *PNAS* 115, no. 5 (2018): 1009-1014. doi: 10.1073/pnas.1717502115. (Source for entire paragraph).

Research into Medical Avenues

GlaxoSmithKline has created a four dose vaccine, RTS,S/AS01, that contains part of the NANP repeats and the C-terminal region from a *P. falciparum* clone of the circumsporozoite protein (CPS) with surface antigens from hepatitis B. The vaccine is currently being implemented in a pilot program in Ghana, Kenya, and Malawi by the Malaria Vaccine Implementation Programme and the WHO. Despite its current use in specific areas in Sub-Saharan Africa, there are some concerns as to its efficacy. One of these concerns is that a parasite introduced with patched CPS haplotype has a 50.3% chance of being fought off the immune system of someone who has received the vaccine, but if the haplotypes are unmatched, that number drops to 33.4%. Part of this may have to do with the fact that the vaccine is based on one parasite clone and is most effective when the invading parasite has the same amino acid sequence.[88]

Phase III clinical trials were conducted in seven Sub-Saharan African countries, looking at the efficacy of RTS,S/AS01 in children enrolled at eleven specific centers in these countries. The researchers found that after 12 months and three doses, the vaccine was 55.8% effective for children aged 5-17 months and 31.3% effective for children aged 6-12 weeks. Protection from the disease was also found for 12 months after the vaccine was administered, but this protection decreased over time. Efficacy was estimated with a negative binomial regression, based on the researchers primary definition of clinical malaria. The researchers defined clinical malaria as "an illness accompanied by an axillary temperature of at least 37.5°C and *P. falciparum* asexual

[88] J Pringle et al. "RTS,S/AS01 malaria vaccine mismatch observed among *Plasmodium falciparum* isolates from southern and central Africa and globally." *Scientific Reports* 8, (2018): 6622. doi: 10.1038/s41598-018-24585-8. (Source for entire paragraph).

parasitaemia or a case of malaria meeting the primary case definition of severe malaria according to a predefined algorithm."[89] In general, this definition is similar to other definitions of malaria, but the algorithm could be brought into question as it's hard to tell if it's the correct algorithm and if it is accurate enough to extrapolate to humans. This study has multiple limitations, including a very specific sample and complicated way of estimating efficacy. It is difficult to extrapolate definitive answers as to this vaccine's efficacy.

Along with research into a vaccine, work has been done on looking into mass drug administration with ivermectin.[90] Ivermectin is an endectocide, commonly used to treat a broad range of parasite infections.[91] Ivermectin is given orally and stays in the blood stream for six days.[92] It has also been used in mass drug administration campaigns in African and Latin America to fight onchocerciasis and lymphatic filariasis.[93] Ivermectin is an agonist of chloride channels in the mosquito that are gated by glutamate, which leads to paralysis and death.[94] It is suggested that Ivermectin can affect the development of asexual blood stages in *P. falciparum*, but more research needs to be conducted to fully understand the interaction.[95] While Ivermectin has been utilized for other diseases, it may be expensive to use to fight malaria, especially on a

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[89] RTS,S Clinical Trials Partnership. "Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomized, controlled trial." *Lancet* 386, no. 9988 (2015): 31-45. doi: 10.1016/S0140-6736(15)60721-8.
[90] T Tizifa et al. "Prevention Efforts for Malaria." *Current Tropical Medicine Reports* 5, no.1 (2018): 41-50. doi: 10.1007/s40475-018-0133-y. (Source for entire paragraph).
[91] Ibid.
[92] Ibid.
[93] YT Pinilla et al. "Promising approach to reducing Malaria transmission by ivermectin: Sporontocidal effect against *Plasmodium vivax* in the South American Vectors *Anopheles aquasalis* and *Anopheles darlingi*." *PLOS Neglected Tropical Diseases* 12, no.2 (2018). doi: 10.1371/journal.pntd.0006221.
[94] Ibid.
[95] Ibid.

broad scale, and must be administered repeatedly as it only stays in the blood stream for a short time.[96]

Resistance in the current antimalarial drug cocktail, ACT, is of concern in the global health community. While failure of ACT treatment has not been seen yet, a mutant allele in the kelch 13 gene in *P. falciparum* has shown reduced susceptibility to ACT. One suggested solution to the possibility of resistance is to add a third drug to the cocktail, thus decreasing the likelihood of resistance. Two drugs and one booster are currently being developed in response to resistance. SJ733 is a drug in early clinical development that is fast acting and even more effective than ACT. It specifically targets the parasite transporter protein. Artefenomel is in Phase IIb of clinical trials, which mimics ACT and contains the endoperoxide bridge that allows ACT to fight parasites. An ACT booster is also being developed that contains analogues of peptides targeted by the parasite, which could circumvent the need for a new drug to be made.[97]

[96] T Tizifa et al. "Prevention Efforts for Malaria." *Current Tropical Medicine Reports* 5, no.1 (2018): 41-50. doi: 10.1007/s40475-018-0133-y.

[97] T Chookajorn. "How to combat emerging artemisinin resistance: Lessons from "The Three Little Pigs"." *PLoS Pathogens* (2018). doi: 10.1371/journal.ppat1006923. (Source for entire paragraph).

Conclusion

Control of malaria is a complex topic that may not be solved anytime soon. Two techniques currently being pushed by the WHO are IRS with pyrethroids and the dispersal of mosquito nets treated with insecticides. Pyrethroid resistance has become abundant across the region through knockdown resistance mutations. This has resulted in a decrease in efficacy of the insecticide, thus pushing for the need of new options for vector control, such as creating a mixture of multiple insecticides for IRS, including DDT. The probability of a mosquito coming into contact with an insecticide is inherently lower due to their timing of their activity, which needs to be taken into consideration in terms of new vector control methods. This highlights the importance of research into effective medical treatments for malaria, as humans can be protected constantly and not just within the confines of their home. As a global community, more needs to be done in terms of research and implementation of elimination methods. Better diagnostic techniques, increased reporting accuracy, and general education about the disease will go a long way to decrease the occurrence of malaria.

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