American Society of Tropical Medicine and Hygiene (ASTMH) 2024 Annual Meeting

November 13-17 New Orleans

Author: Nuria Ena Valls, Hospital General Universitario de Elche.

What is the ASTMH

The American Society of Tropical Medicine and Hygiene, is the largest international scientific organization of experts dedicated to reducing the worldwide burden of tropical infectious diseases and improving global health. The current organization was formed in 1951 with the amalgamation of the American Society of Tropical Medicine, founded in 1903, and the National Malaria Society, founded in 1940.

They accomplish this through generating and sharing scientific evidence, informing health policies and practices, fostering career development, recognizing excellence, and advocating for investment in tropical medicine/global health research.

Their goals include:

- **Advancing research** in tropical diseases
- **Supporting career development** in tropical medicine/global health
- **Fostering international scientific collaboration**
- **Educating medical professionals, policymakers and the public** about tropical medicine/global health
- **Recognizing exceptional achievement** in tropical medicine/global health
- **Promoting science-based policy** regarding tropical medicine/global health

About New Orleans

New Orleans (commonly known as NOLA or The Big Easy among other nicknames) was Founded in 1718 by French colonists and was once the territorial capital of French Louisiana before becoming part of the United States in the Louisiana Purchase of 1803. It is located along the Mississippi River in the southeastern region of the U.S. state of Louisiana and with a population of 383,997 according to the 2020 U.S. census, it is the most populous city in Louisiana. Serving as a major port, New Orleans is considered an economic and commercial hub for the broader Gulf Coast region of the United States.

New Orleans is world-renowned for its distinctive music, Creole cuisine, unique dialects, and its annual celebrations and festivals, most notably Mardi Gras.

Tropical Medicine in New Orleans

New Orleans is know as a city with a lot of floklore around vampires and death. This is most propably related to the many Yellow fever outbreaks that have ravaged the city throughout the 18th and 19th century.

The first recorded outbreak dates back to 1796, with the worst outbreak reccorded in 1834, when 8,000 of the city's residents died. Subsequent outbreaks occurred almost annually, leading to a total of approximately 41,000 deaths in the years that followed, until 1905, the year of the last recorded epidemic. The disease is transmitted by the female *Aedes aegypti* mosquito, a species native to West Africa. It is believed to have been introduced to New Orleans through banana merchant ships involved in trade.

Surrounded by the Missisipi river to the south and the Ponchantrain Lake to the north, combined with the widespread use of wooden water tanks for storing drinking water, the metropolis became an ideal breeding ground for the mosquito and the disease it carried.

In response to the many deaths, seven physicians, none older than 26, founded a medical college in New Orleans to provide training in the pressing health issues affecting the region, including malaria, yellow fever, small pox, and cholera which later became the first Tropical Medicine School of the United States.

Updates on Tropical Medicine

1. Malaria emerging technology

Mosquitoes modified with gene drive systems are being proposed as new tools that will complement current practices aimed at reducing or preventing transmission of vector-borne diseases such as malaria. Gene drive technology offers the promise for a high-impact, cost-effective, and durable method to control malaria transmission that would make a significant contribution to elimination. Gene drive systems have the potential to spread new genetic traits through interbreeding populations of malaria mosquitoes from low initial introductions. The envisioned goal for applying this technology is to reduce or eliminate vector mosquito populations or, alternatively, to render them less competent to transmit pathogens. . This is achieved using the **CRISPR/Cas9 system**, a versatile molecular tool that edits DNA by utilizing *clustered regularly interspaced short palindromic repeats (CRISPR)* and associated protein 9 (Cas9).

Strategies for Gene Drive Applications

1. **Population Suppression**:
   Aims to reduce mosquito populations below the threshold needed for malaria transmission, without necessarily eradicating them. This involves gene knock-out

techniques targeting survival, reproduction, or fertility. For instance, reducing female progeny or shifting the sex ratio toward males can significantly suppress populations.

2. **Population Replacement**:
   Seeks to decrease the ability of mosquitoes to transmit malaria by:
   - Inactivating genes that enable parasite survival or human feeding.
   - Introducing new genes that produce molecules lethal to the malaria parasite.
     Success depends on these modifications being tightly linked to the gene drive system to ensure their spread and persistence in mosquito populations.

What is CRISPR/Cas9

CRISPR gene editing (pronounced /ˈkrɪspər/ "crisper"), an acronym for "Clustered Regularly Interspaced Short Palindromic Repeats", is a genetic engineering technique in molecular biology that allows for the modification of the genomes of living organisms. It is based on a simplified version of the bacterial antiviral defense system CRISPR-Cas9.

The CRISPR-Cas9 system functions as a defense mechanism against viruses, enabling bacteria to "remember" and attack viruses that have previously infected them. Acting like genetic scissors, the Cas9 nuclease cuts both strands of the target DNA sequence to introduce modifications through one of two methods, incorporating fragments of viral DNA into their genome in a region known as CRISPR. These fragments serve as a "memory library" of past infections.

Francisco Juan Martínez Mojica, a scientist from Elche, was the first (in 2005) to suggest that these sequences could be related to bacterial immunity against attacks by certain viruses.

Real applications:

A) **"TP13" Study (Carballar-Lejarazú et al., 2023)**:

This article describes the development and evaluation of a CRISPR-Cas9-based gene-editing system designed to modify populations of *Anopheles gambiae and Anopheles gambiae coluzzi*, in order to reduce or prevent malaria transmision.

The main objective was to design a gene drive system that introduced monoclonal antibodies m1C3 and m2A10 targeting parasite ookinetes and sporozoites respectively in the African malaria mosquitoes *Anopheles gambiae* (AgTP13) and *Anopheles coluzzii* (AcTP13) reducing parasite prevalence and intensities of infection.

Importance of the m1C3 and m2A10 antibodies: The proportion of *infected* mosquitoes isn't equivalent to the proportion of *infectious* mosquitoes; it has been demonstrated that levels of <10,000 sporozoites in mosquito salivary glands significantly decreased the probability of subsequent infection. Furthermore, the likelihood of a *Plasmodium*-infected mosquito taking multiple blood meals may be higher than that of an uninfected mosquito as the parasite increases the mosquito's metabolic demand leading to a state of nutrient deficiency, can manipulate the mosquito's sensory and neural pathways, and impairs the mosquito's ability to fully engorge during a blood meal, possibly due to interference with salivary gland function which prompt the mosquito to seek additional blood meals to compensate. Finally m1C3 and m2A10 antibodies are regulated respectively by the *A. gambiae Carboxypeptidase A* [*AgCPA*,

(Cp)] which activates the m1C3 antibody gene in the midgut and *A. stephensi Vitellogenin 1* [*AsVg1*, (Vg)] which induces the expression of m2A10 in other tissues after the development of sporozoites.

Results: Both AgTP13 hemi- and homozygous adult females exhibited strong parasite suppression with oocyst prevalence reduced from ~74% in controls to 44.2% (hemizygotes, ~40% reduction) and 33.9% (homozygotes, ~54% reduction) and significant reductions in the prevalence and MII of salivary gland sporozoites. Prevalence was reduced from 73 to 75% in controls to 42% (hemizygotes, ~43% reduction) and 31% (homozygotes, ~58% reduction). Computational models predicted that the release of these mosquitoes could reduce human malaria incidence by 50-90% within 1-2 months under ideal conditions. A reduction of ≥90% in incidence could be sustained for years, depending on factors such as the genetic resistance load and the effectiveness of the system in the field.

Conclusion: The study concludes that these genetic modification systems are promising for malaria control, although additional validations are required under field conditions.

B) **CRISPR/Cas9 and the *doublesex gene* (Kyrou et al., 2018)**:

This article describes the development and evaluation of a CRISPR-Cas9-based gene-editing system designed to suppress populations of *Anopheles gambiae*, the primary vector of malaria.

The main objective was to design a gene drive system that disrupts sexual differentiation in mosquitoes, specifically targeting the *doublesex (dsx)* gene, to drastically reduce the reproductive capacity of *A. gambiae* populations.

Importance of the *doublesex* (dsx) gene: The *dsx* gene encodes two alternative transcripts: *dsx-female (AgdsxF)* and *dsx-male (AgdsxM)*, which control sexual differentiation in *A. gambiae*. The female isoform (*dsxF*), includes a specific exon (exon 5), which is highly conserved among *Anopheles* species and essential for female fertility and that can be targeted with the CRISPR-Cas9 system, blocking the formation of the functional *dsxF* transcript. This modification did not affect male development or fertility, but homozygous females carrying the mutated allele exhibited an intersex phenotype and complete sterility.

Results: In controlled experiments, the gene drive reached 100% prevalence within 7–11 generations, resulting in a progressive reduction in egg production and ultimately, total population collapse. Heterozygous females showed reduced fertility, while homozygous females were unable to feed on blood and did not produce eggs. Anomalies in sexual morphology were observed only in dsxF−/− genetic female mosquitoes. This group of XX individuals showed male-specific traits, including a plumose antenna (red arrowhead) and claspers (blue arrowheads). This group also showed anomalies in the proboscis and accordingly they could not bite and feed on blood. Representative samples of each genotype are shown. (b) Magnification of the external genitalia. All dsxF−/− females carried claspers, a malespecific characteristic. The claspers were dorsally rotated rather than in the normal ventral position.

Conclusion: The gene drive system targeting the *doublesex* gene in *A. gambiae* shows promise as a tool for suppressing populations of malaria vector mosquitoes. Its demonstrated effectiveness in causing population collapse under laboratory conditions suggests significant potential, although careful risk-benefit evaluation will be required before field implementation.

2. Clinical partial resistance to artemisins in malaria

Conrad, *et al.* NEJM (2023)

Partial resistance of *Plasmodium falciparum* to the artemisinin component of artemisinin-based combination therapies (ACTs) defined as delayed clearance after therapy (where patients take longer to achieve a negative blood smear despite receiving appropriate dosing often defined as >72 hours for clearance) emerged in Southeast Asia and is mediated mainly by mutations in the kelch protein K13 (PfK13). Clear evidence of artemisin partial resistance has now been identified in the Greater Mekong subregion (GMS) and Africa – specifically in Eritrea, Rwanda and Uganda.

Platon, L. Trends in Parasitology (2024)

The importance of the ring-stage and artemisin resistance: Artemisinin acts by generating reactive oxygen species (ROS) and alkylating essential parasite proteins, ultimately leading to parasite death. It is particularly effective against young ring-stage parasites, the first stage of the intraerythrocytic developmental cycle (IDC) following merozoite invasion of erythrocytes. During the ring stage, parasites can develop into either asexual or sexual stages, or pause their growth under unfavorable conditions.

The ring stage demonstrates remarkable plasticity in response to adverse conditions, such as exposure to artemisinin. *Plasmodium falciparum* has evolved resistance to nearly all antimalarial drugs through genomic adaptations and nonspecific stress-response mechanisms, including ring-stage temporary growth arrest (TGA). This TGA mechanism allows parasites to enter a state of metabolic quiescence, providing passive resistance to various stressors and ensuring the survival of a subset of the population within the host. In artemisinin-sensitive strains, ACTs are highly effective against ring-stage malaria parasites, resulting in rapid parasite clearance. However, in resistant strains carrying *PfKelch13* mutations, artemisinin is less effective at eliminating ring-stage parasites due to their ability to temporarily halt growth and evade drug action.

Conrad, M.D. *et al* have studied the evolution of Partial Resistance to Artemisinins in Malaria Parasites in Uganda. By 2021–2022, the prevalence of parasites with resistance markers reached more than 20% in 11 of the 16 districts where surveillance was conducted. The PfK13 469Y and 675V mutations were seen in far northern Uganda in 2016–2017 and increased and spreaded, reaching a combined prevalence of 10 to 54% across much of northern Uganda, regions. In conclusión; data from Uganda showed the emergence of partial resistance to artemisinins in multiple geographic locations, with increasing prevalence and regional spread over time.

Visser MT et al, Trop Med Int Health (2022)

Implications: Treatment guidelines for uncomplicated *Plasmodium falciparum* malaria in non-endemic countries generally follow WHO recommendations, which advocate for 3-day courses of Artemisinin-based Combination Therapies (ACTs) as the standard regimen. However, these guidelines often fail to address the risk of drug resistance in returning travelers.

ACTs should be the first-line treatment for all uncomplicated malaria cases, but the selection of a specific ACT should consider regional resistance patterns. In particular, travelers returning from the Greater Mekong Subregion (GMS), where high rates of treatment failure have been reported, require special attention. In a recent prospective study, conventional treatment with dihydroartemisinin–piperaquine showed therapy failure rates of up to 93% in the GMS. As a result, patients returning from this region could be treated with artesunate–mefloquine or artemether–lumefantrine. Additionally, these patients should be monitored closely due to the increased likelihood of treatment failure. In areas with established resistance, extending the duration of ACT regimens has been shown to improve cure rates.

Retrospective studies from Sweden and the Czech Republic further underscore the limitations of the standard 3-day artemether-lumefantrine (A/L) regimen. Late treatment failure rates among returning travelers ranged from 5.3% to 13.9%, whereas no failures were reported with alternative regimens, such as mefloquine or atovaquone-proguanil. Nonimmune patients—such as travelers from non-endemic regions who lack acquired partial immunity—are particularly vulnerable to treatment failure. This vulnerability is likely due to suboptimal lumefantrine plasma levels, which contribute to the observed high failure rates in European patients.

Tun, K.M et al *Malar J* (2018).

Given these considerations, the routine use of the standard 3-day A/L regimen in nonimmune travelers should be critically re-evaluated. Extended A/L regimens lasting 5 days have been shown to be both safe and more effective, offering a viable alternative for ensuring adequate treatment outcomes in these patients.

3. <u>False negative results using rapid diagnostic testing due to HRP2/3 mutations.</u>

Prosser C et al Emerg Infect Dis. (2021).

The diagnosis of malaria primarily relies on the visualization of the parasite in a blood smear, which must be prepared and evaluated immediately by experienced personnel. This method remains the gold standard due to its high sensitivity and specificity when performed correctly.

In the absence of microscopy experts, serological tests, such as antigen detection tests based on the histidine-rich protein 2 (HRP-2) of *Plasmodium falciparum*, are used. These tests are highly specific and sensitive but have significant limitations:

- They only detect *P. falciparum*.
- Their sensitivity decreases in cases of low parasitemia.

In remote or resource-limited areas, rapid diagnostic tests (RDTs) have become an essential tool for malaria case management. These tests are affordable, easy to transport and store, and require less technical expertise compared to microscopy, while offering comparable sensitivity to high-quality microscopy.

HRP-2-based RDTs detect the HRP-2 antigen (and, to a lesser extent, HRP-3 due to cross-reactivity) at concentrations as low as 1 ng/mL of blood. However, in practice, their detection limit is similar to that of high-quality microscopy (~200 parasites/µL). A critical limitation of these RDTs is the deletion of the *pfhrp2* and *pfhrp3* genes, which can lead to false-negative results. Reports of *pfhrp2*-deleted parasites have been documented in several

African countries (including Nigeria, Sudan, and South Sudan), as well as in India, China, Myanmar, and some South American countries, such as Peru.

The presence of a single *pfhrp2* deletion increases the risk of RDT failure, particularly in cases of low parasitemia or when substandard RDTs are used. In cases of double deletions of *pfhrp2* and *pfhrp3*, parasites become undetectable by HRP-2-based RDTs, posing a significant public health threat in regions where the prevalence of these deletions exceeds 5%.

An analysis of imported *P. falciparum* cases revealed *pfhrp2* and *pfhrp3* deletions in 12 countries, with levels exceeding 10% in samples from Nigeria, Sudan, and South Sudan, raising serious concerns about the efficacy of HRP-2-based RDTs in these areas. Retrospective studies showed consistent results between SD BioLine and CareStart HRP-2-based tests, whereas BinaxNOW™ exhibited lower sensitivity in some instances.

The BinaxNOW™ Malaria test is an in vitro immunochromatographic assay designed to detect *Plasmodium* antigens in venous or capillary whole blood. It targets the HRP-2 antigen of *P. falciparum* and a panmalarial antigen common to the four malaria species that infect humans (*P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*).

In conclusion, while RDTs have transformed malaria diagnosis in resource-limited settings, the increasing prevalence of *pfhrp2* and *pfhrp3* deletions underscores the need for complementary diagnostic strategies to ensure accurate detection and avoid treatment failures. In regions where these deletions are prevalent, a combined approach is recommended, incorporating high-quality microscopy, alternative RDTs based on pLDH or aldolase, and molecular diagnostic tools. Strengthening epidemiological surveillance and adapting national policies are also critical to addressing these challenges and maintaining reliable malaria diagnosis.

Furthermore, given the practical challenges of microscopy in rural and low-resource areas, when the prevalence of false-negative RDT results caused by *pfhrp2* deletions exceeds 5%, the benefits of switching to alternative RDTs, such as Pf-LDH-based tests, often outweigh their slightly lower sensitivity. This strategic shift can mitigate the risks associated with *pfhrp2* deletions while ensuring continuity in malaria case management.

4. Oropuche virus and its impact in Spain.

Discovered in 1955 in Vega de Oropuche, Trinidad and Tobago, Oropouche fever has since caused outbreaks across South and Central America, with over 500,000 cases reported to date. The disease is caused by the Oropouche virus (OROV), a member of the *Bunyavirales* order, characterized by a negative-sense, single-stranded RNA genome enveloped in a spherical lipid structure. Its primary vector is *Culicoides paraensis*, though mosquitoes like *Culex quinquefasciatus* and *Aedes aegypti* can occasionally transmit it. The virus circulates in sylvatic cycles involving birds, non-human primates, and sloths, while urban transmission occurs in humans.

Clinically, Oropouche fever presents as an acute febrile illness with headache, muscle, and joint pain. About 60% of patients experience a recurrence of symptoms 1–2 weeks post-recovery. Severe cases can involve hemorrhagic manifestations and central nervous system (CNS) symptoms, although recovery is typically complete without long-term sequelae. In 2024, two deaths in previously healthy adults and concerns about fetal complications like stillbirth, miscarriage, and microcephaly highlighted the need for further investigation.

In 2024, the Pan American Health Organization (PAHO) and the World Health Organization (WHO) issued an epidemiological alert following 7,700 cases reported in Latin America by August 1. Notably, 24 imported cases were detected in Europe, 12 in Spain. Although the primary vectors are absent in Europe, the outbreaks underscore the importance of vigilant surveillance and research into the disease's potential complications.