Targeting the Parasite within the Vector: Exploring Novel Approaches to Prevent Transmission of Vector-Borne Diseases

A Virtual Workshop Sponsored by the
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Meeting Report

NIH National Institute of Allergy and Infectious Diseases

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Executive Summary

Organizers from the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health brought together experts in vector biology, parasitology, and related areas to discuss approaches to interrupt parasite development within the arthropod host and prevent transmission of human vector-borne diseases. In accordance with NIAID's goals, the virtual workshop promoted communication and multidisciplinary collaboration among researchers, highlighted existing and needed resources, and sparked ideas about accelerating research. Lee Hall, Chief of DMID's Parasitology and International Programs Branch, opened the workshop and outlined his vision that the meeting would lay the foundation for the development of interventions and ultimately accelerate the needed public health mechanisms to address the burden of vector-borne disease.

On day one of the workshop, participants heard oral presentations on the state of the science for four parasite/vector systems: trypanosome/tsetse fly, trypanosome/triatomine bug, *Plasmodium*/mosquito, and *Leishmania*/sand fly. Additional presentations covered various approaches to targeting the parasite within the vector and tools available to accelerate research. On day two, participants met in breakout sessions to identify limitations, opportunities, mechanisms for enhancing target discovery, and research priorities for the four parasite/vector systems discussed on day one, plus tick-borne diseases.

The breakout sessions yielded numerous suggestions for advancing research. Four cross-cutting points emerged:

- Core facilities are needed to generate reagents and organisms (pathogens and vectors) to increase consistency and reproducibility.
- The field of vector/pathogen interactions needs new ideas from a broader investigator pool with different perspectives. A web-based platform for linking investigators across research specialties might be a good start to enable multidisciplinary collaborations and facilitate the exchange of ideas.
- Better information and data management and sharing are needed across research fields.

Prior to the workshop, a communication platform was established to encourage participants to exchange ideas, information, and resources. The tool will be maintained to encourage continued collaboration among experts from the many different disciplines involved in targeting the pathogen within the arthropod vector.

Meeting Summary

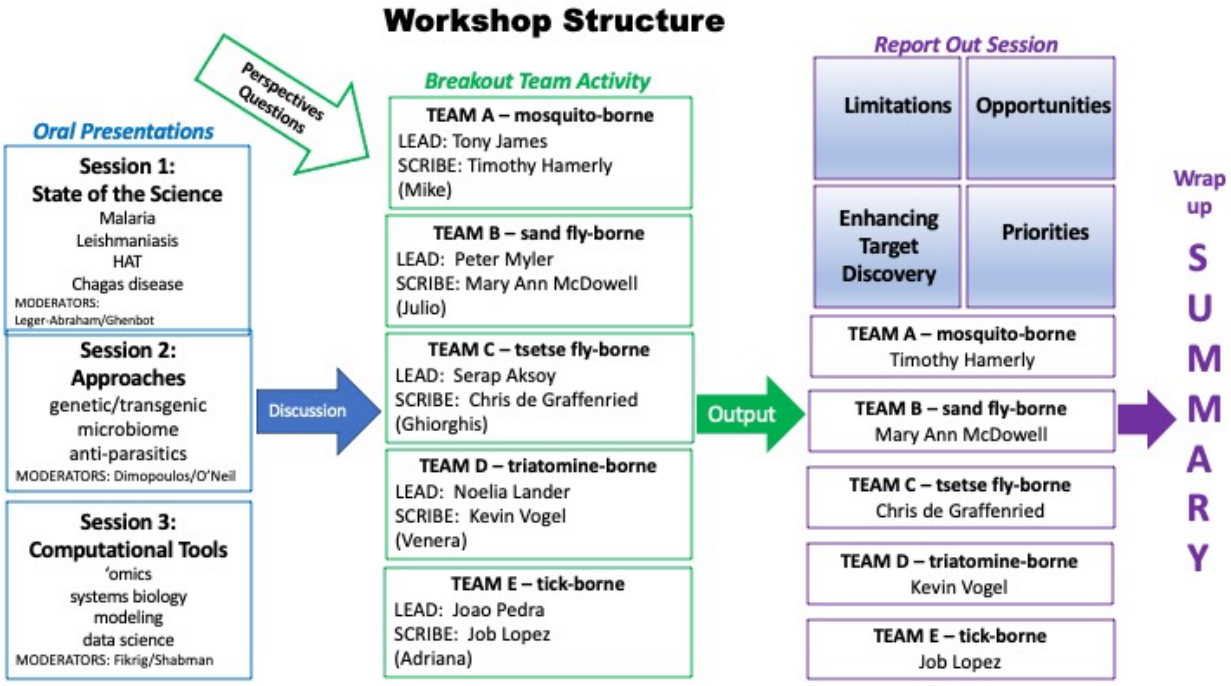
Introduction and Purpose

Organizers from the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH) brought together experts in vector biology, parasitology, and related areas to discuss approaches to interrupt parasite development within the arthropod host and prevent transmission of human vector-borne diseases. The goals of the workshop were as follows:

- Promote communication and collaboration among researchers
- Highlight existing resources and tools available
- Identify additional resources and tools needed
- Spark ideas to accelerate research and ultimately prevent transmission of vector-borne pathogens

Day one of the workshop consisted of oral presentations on the state of the science for four parasite/vector systems: trypanosome/tsetse fly, trypanosome/triatomine bug, *Plasmodium*/mosquito, and *Leishmania*/sand fly. Additional presentations covered various approaches to targeting the parasite within the vector and tools available to accelerate research. On day two, participants met in breakout sessions to identify limitations, opportunities, mechanisms for enhancing target discovery, and research priorities for the four parasite/vector dyads discussed on day one, plus ticks and tick-borne diseases (See Figure 1).

Figure 1: Workshop Organization



The list of participants appears in Appendix A. Resources and background materials can be found in Appendix B. A list of selected key publications can be found in Appendix C.

Remarks from Lee Hall, Chief, Parasitology and International Programs Branch, DMID, NIAID

Dr. Hall noted that according to the World Health Organization, vector-borne diseases account for 17 percent of all infectious diseases and cause more than 700,000 deaths annually, posing a substantial burden on society. More effective interventions could have a significant impact on morbidity and mortality. For many of these diseases, new interventions and approaches are needed.

This workshop offered an opportunity to address the perception of vectors as "flying syringes." Researchers in the field recognize that vectors are not just passive reservoirs or transporters but rather are crucial sites for pathogen development and replication. The complex processes involved in pathogen development offer many potential targets for intervention. Dr. Hall hoped that the field would capitalize on recent advances in science to devise novel approaches to vector-borne disease research, such as gene editing, structure-based design, and influencing the microbiome, as well as the use of modern computational tools, systems biology, data science, and modeling. He anticipated that this workshop would lay the foundation for the development of interventions and ultimately accelerate the needed public health mechanisms to address the burden of vector-borne disease.

Dr. Hall encouraged participants to take advantage of the workshop to engage with peers, seek out collaborations, and generate new insights.

Session 1: State of the Science for Parasite/Vector Systems

Each speaker gave a brief presentation (either prerecorded or live) on interactions, focusing either on the role of the vector or the parasite. Following presentations, participants posed questions for discussion. All questions and responses are combined in this summary.

Trypanosome/Tsetse Fly Interactions

Vector Speaker: Serap Aksoy, Yale University

Diseases transmitted by tsetse flies cause significant harm in animals and remain a persistent threat for humans. Vector control is highly effective in reducing disease. Parasites undergo three major developmental events within the tsetse fly, and, with each event, the efficiency of transmission of the parasite decreases. There are barriers to invasion of the tsetse fly at these events: the peritrophic matrix; the midgut colonization; and the transmission of infectious material into the salivary gland.

Some genomic data exist for tsetse flies and trypanosomes. Dr. Aksoy called for better curation of existing genomics resources, validation of single nucleotide polymorphism discovery data, and expanded knowledge about the contributions of microbial partners. She noted that paratransgenesis is a fast, effective alternative to transgenesis. Efforts should be made to improve maternal transmission efficiency of transgenic symbionts to establish paratransgenic tsetse lines.

Most functional genomics data come from laboratory lines of tsetse flies, which are limited by genetic bottlenecks that result from inbreeding. Investigations with natural populations and field-

driven colonies are needed. Finally, parasite transgenesis is feasible, and the field should explore how to improve the ability of flies to transmit transgenic parasites.

Parasite Speaker: Christian Tschudi, Yale University

Dr. Tschudi summarized the history of the science, dating back to the 1903 demonstration of the link between trypanosomiasis and tsetse flies and continuing to recent technological advances that revolutionized current understanding of the relationship between the arthropod vector and its vertebrate host, including genome-wide RNA interference (RNAi), single-cell RNA sequencing (scRNA-seq), high-throughput phenotyping, and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9). Using these technologies, investigators have shed light on trypanosome development within the tsetse fly and the expression of key proteins that cause infection in hosts.

Among the outstanding issues and questions for the field are the following:

- Genetic exchange takes place, but there are knowledge gaps around the details of the second meiotic division, nuclear and kinetoplast DNA exchange, and zygote formation.
- The mechanics of DNA exchange await elucidation.
- Does social motility take place during peritrophic matrix crossing or migration to the proventriculus?
- What physical or chemical cues guide the different migration patterns of *Trypanosoma brucei* in the fly?
- What are the receptors involved in the attachment of epimastigotes to the tsetse salivary gland epithelial cells?
- What signal(s) initiates trypanosome movement and differentiation during the developmental cycle in the tsetse?

Discussion

It was asked whether the trypanosomes change surface coats while they are in the tsetse gut. Dr. Tschudi said the trypanosomes are taken up by the mammalian host, and they carry the variant surface glycoprotein coat. As soon as they enter the midgut, they shed the coat and put on a new one, which is procyclin, becoming noninfective during that process. Dr. Aksoy said the mammalian parasite rapidly changes its coat to the procyclic stage in the vector, typically within 12 hours. Then the metacyclic parasite, once introduced into the mammalian bite site, also undergoes a change to express mammalian variant surface glycoproteins. It takes 36 to 48 hours before the process is initiated at the bite site, so there is a short window to interfere with the metacyclic parasites at the bite site. Dr. Tschudi added that, from live cell imaging, it appears that metacyclic variant surface glycoprotein expression is rapid and only occurs for about 5 hours (in an in vitro system), which is surprising.

There was a discussion about whether any specific motif has been recognized from the genetic transcriptions that can identify the mechanism by which RBP6 mutates the expression of genes. Dr. Tschudi responded that the targets of RBP6 on messenger RNA have been identified. There are about 1,000 of them, across multiple categories, including metabolism, mitochondria, and mitogenesis. RBP6 is a master regulator that mainly regulates at the level of transcription and translation.

Participants also asked about the possibility of developing vaccines against the trypanosome pathogen while it is still in the tsetse gut. Dr. Aksoy responded that transmission-blocking vaccines have been explored in various systems, and an anti-procyclin vaccine could be pursued, although whether such a vaccine can be developed remains an open question. Dr. Aksoy noted that vaccine antibodies would be expected to survive, pass the peritrophic matrix barrier, and be effective against procyclic infections in the gut. Given new technology, such as paratransgenic symbiotic systems, the topic might be worth further discussion, Dr. Aksoy said.

Trypanosome/Triatomine Bug Interactions

Vector Speakers: Lori Stevens and Raquel A. Lima-Cordon, University of Vermont

Innovative genetic analysis techniques combined with liquid chromatography tandem mass spectrometry (LC-MS/MS) confirm that the Triatominae abdomen is a rich source of information about the vector, parasite, microbiome, and vertebrate blood meal source that can be used to develop antiparasitics and transgenics. For example, research demonstrates that the richness and community structure of the microbiome vary between infected and uninfected vectors. However, more work is needed to investigate the relationships among the transcriptome, microbiome, parasite virulence, and vector competence. Furthermore, the ability to identify multiple blood meal sources accurately using LC-MS/MS could improve vector control strategies.

For Chagas disease, questions remain about how much covariation exists within the parasite, vector, vertebrate host, and microbiome, but the field lacks enough specimens to achieve adequate statistical power in analysis. Researchers developed a method for extracting DNA from preserved museum specimens going back to 1935 that yield complete mitochondrial vector phylogenies as well as parasite genomics, which could be used to address questions around covariation.

Parasite Speaker: Eric Dumonteil, Tulane University

From DNA extracted from the abdomen of the triatomine bug, investigators can create a profile that includes species identity and population genetics, blood meal sources, parasite genotypes, and microbiome factors. Better profiles and genotyping should illuminate what is unique to a geographic site that explains differences in prevalence, which could then be targeted for intervention.

A profile of *Triatoma dimidiata* shows that the bug feeds on multiple vertebrate hosts and switches hosts frequently, providing many opportunities to transmit the parasite across multiple species. This information can be further mined to reconstruct feeding networks and determine the pathways for transmission of disease. These data will inform models that can show the potential effects of intervention. For example, reducing infection in chickens or dogs would reduce human infection substantially, and reducing infections in both chickens and dogs benefits humans even more. Work is underway to expand the concept of manipulating the host community. In addition, research is seeking out bacteria that could make triatomine bugs refractory to *Trypanosoma cruzi* infection, an approach suggested 20 years ago but made possible by current tools that allow researchers to understand the interactions and opportunities and to break the cycle of infection.

Discussion

A participant noted that Dr. Dumonteil's feeding profile data could be biased by collection biases, because the bugs are mostly collected from human habitats.

It was asked whether Dr. Stevens' laboratory has detected triatomine bugs infected with *Trypanosoma rangeli*. A response was given that the parasite had been detected but is more commonly found in *Rhodnius* species.

There was a discussion on zooprophylaxis as a potentially good approach. The drawback is the increase in the local vector population in the area of transmission. The greater the vector population, the greater the biting rates and the greater the risk. It was asked how to reduce that potential negative effect. Dr. Dumonteil responded that feeding profiles vary a lot according to habitat, and bugs from human habitats are indeed biased toward humans and domestic species (which are the most important species from an epidemiologic perspective). However, he and his colleagues are also investigating sylvatic populations to understand the zoonotic cycles. Dr. Dumonteil added that his team is using modeling to assess the conditions under which zooprophylaxis might work.

Malaria Parasite/Mosquito Interactions

Vector Speaker: Marcelo Jacobs-Lorena, Johns Hopkins University

The midgut lumen of the mosquito is the best target for interventions to prevent transmission of pathogens because it produces the most sporozoites following ingestion of gametocytes. The first step toward genetic modification of mosquitoes to secrete compounds that kill the parasite (transgenesis) has been accomplished successfully in the laboratory. The use of CRISPR/Cas9 to create gene drives has been effective in laboratories but not has not yet been tested in the field.

Alternatively, paratransgenesis results in creation of bacteria engineered to secrete compounds in the gut of the mosquito that kill the parasite. When a mosquito feeds, the number of bacteria in the gut increases by 100-fold, so killing the parasite in the gut would ultimately limit the transmission of the parasite at the next feeding. Researchers have demonstrated that engineered bacteria can spread rapidly through horizontal and vertical transmission in the vector population. Transgenesis is more effective than paratransgenesis, but a combination of the two will likely be the most successful approach.

Parasite Speaker: Ashley Vaughan, Seattle Children's Hospital

The mosquito bloodmeal is vital for oocyst growth. Neither the parasite nor the mosquito can synthesize isoleucine. Optimal oocyst growth requires constant nutritional intake by the mosquito. The hormone 20-hydroxyecdysone plays an essential role, with mosquito hormone signaling coregulating both egg and parasite development. Knocking down heterodimer ecdysone receptor results in fewer oocytes, but they are larger and develop more quickly. Lipids are abundant after a blood meal and can be accessed by the developing oocyte as it matures. Parasite development requires mitochondrial respiration, heme biosynthesis, and fatty acid biosynthesis.

Dr. Vaughan summarized that *Plasmodium falciparum* oocyst development requires uptake of nutrients associated with the blood meal. Oocysts likely scavenge nutrients from the hemolymph.

P. falciparum oocysts require de novo fatty acid synthesis, heme biosynthesis, and mitochondrial respiration for sporozoite production. An important question to explore is whether oocysts sense nutrient levels and enter a dormant state as needed. Dr. Vaughn noted that in vitro sporozoite production is possible but still challenging. In vitro mechanisms are needed to advance research.

Discussion

It was asked whether releasing infected mosquitoes into the field could introduce a modified bacteria into the environment. Dr. Jacobs-Lorena acknowledged that paratransgenesis and gene drives introduce genetically modified organisms into nature, which has social consequences and raises regulatory issues. In a major development, *Serratia* bacteria were isolated from mosquitoes in the field that naturally impair parasite development. The *Serratia* bacteria spread through the mosquito without genetic modification, and therefore offer an approach that would make paratransgenesis easier to test and, eventually, to implement. It was asked whether mosquitoes could develop resistance to the bacteria. Dr. Jacobs-Lorena replied that the *Serratia* bacteria have a symbiotic relationship with the mosquito, with no fitness cost to the mosquito, so resistance is unlikely to develop.

Participants also asked whether any marker exists that could be used to enable paratransgenesis in the sand fly. Dr. Jacobs-Lorena said that fluorescence protein genes are introduced into bacteria in the laboratory as markers and are easy to follow. In a field trial, fluorescent genes could be added to the modified bacteria released to track their progression.

Participants asked whether paratransgenesis directed against *Plasmodium* influences the vector competence of mosquitoes for other pathogens (e.g., arboviruses). Dr. Jacobs-Lorena did not think so, because the effector molecules that are secreted by the bacteria target the parasites directly. It could target other parasites, but *Anopheline* mosquitoes transmit very few other pathogens besides *Plasmodium*. Dr. Jacobs-Lorena did not think paratransgenesis changed the physiology of the mosquitoes. If anything, it would impair transmission of other pathogens, not facilitate it, he said.

There was a discussion as to whether the *Serratia* bacteria colonize the salivary glands of the mosquito and could be transmitted to mammals during bites. Dr. Jacobs-Lorena said experiments were conducted with a sensitive test to see whether, after feeding on *Serratia*, mosquitoes could release bacteria into the blood meal. The results determined that the bacteria do not go through the salivary gland and are not present in the lumen of the salivary gland, so the use of *Serratia* is safe for mammals.

It was asked whether atovaquone should be added to other antimalarials to protect against transmission even if it does not cure people immediately. Dr. Vaughan noted that atovaquone is still used in combination with other drugs as a first-line antimalarial defense. It is also effective against malaria at the liver stage.

Participants also asked whether in Fas2 knockouts, sporozoites fail to develop properly in the midgut, fail to migrate to the salivary glands, or fail to survive in the salivary glands. Dr. Vaughan responded that the *P. falciparum* sporozoites fail to develop within the oocyst, although the oocyst does undergo development. He believes that the fatty acids are essential for the

formation of the sporozoite membranes, although he has no direct evidence of this. Interestingly, rodent malarias do not require Fas2 during mosquito stage development.

Leishmania/Sand Fly Interactions

Parasite Speaker: Steve Beverley, Washington University

Lipophosphoglycan (LPG) plays a key role in the sand fly vector, and modifying LPG reveals some unexpected consequences. Not all species use LPG for binding. Better understanding is needed about the relevant parasite/sand fly ligands and receptors. The role of proteophosphoglycan in transmission from the fly to the mammalian host and its role in the mammalian host should also be elucidated. Research is needed to determine whether endogenous microbiota could be engineered to express LPG, which could block binding. Sand flies are not widely accessible, so alternatives and model systems would be helpful, such as *Drosophila* flies or cell lines, sand fly cell lines, and midgut organoid cultures.

The future of research most likely lies in advanced technology that allows investigators to look at many genes at once in difficult-to-assay systems. Individual laboratories often do not have the resources to take on such big efforts, so the field would benefit from consortia that share resources with the community.

Genetic crossing is key to fly biology and is a fundamental mechanism of genetic diversity. *Leishmania* crossing occurs with sufficient frequency to generate diversity but infrequently enough to maintain successful lineages. Crossing could potentially be used for positional cloning of complex phenotypes—for example, to identify tropisms in sand flies or human hosts. However, this approach requires flies, so alternatives should be explored. One laboratory has had some success with *in vitro* crossing using *Leishmania tropica*. It is not clear how well the approach mimics real crossing, but that might not matter for genetic mapping and could open doors in parasite and vector biology research.

More investigation is needed to understand the impact of *Leishmania* on the fly, particularly the impact on metabolism, the fly's immune responses, and the role of glycoconjugates. Until recently, the creation of sand fly mutants was considered impossible, but it has been achieved via CRISPR/Cas9. Maintaining mutant lines in culture is very challenging, especially for sand flies.

Vector Speaker: Tiago D. Serafim, Division of Intramural Research, NIAID

More is known about the parasite than the vector, particularly how the vector succeeds as an infectant. The sand fly feeds every 5–6 days. With each blood meal, the parasite multiplies in the midgut. The sand fly only becomes capable of transmitting infection after the second blood meal. The demonstration of a shortened parasite lifecycle that enhances infectivity with every successive meal is important not only for understanding *Leishmania* species in the sand fly but also applies to mosquitoes' transmission of virus, as demonstrated by other investigators.

Dr. Serafim described research on the role of the microbiota. His mentioned that his laboratory found that the sand fly microbiome is important for the sustainability of the parasite within the vector.

Discussion
Participants asked how metacyclics in the sand fly gut deal with the metabolites in the blood meal, as they are exposed to them multiple times. Dr. Serafim said there are no data to answer the question.

There was a discussion as to whether there is any knowledge about *Leishmania* parasite stage modulation in response to the sand fly midgut microbiota and what is known about the dynamics of microbiota composition and diversity in response to consequent blood meals and their correlation with parasite stages. Dr. Serafim responded that the parasite does regulate; there is selection of some families of sporozoites that the infection progression generates, so the gut loses diversity. Which form performs this selection, lowering the amount of bacteria, is not known, only that the progression generates it. Regarding metabolites, when the bacteria are extracted and the parasite dies, it is possible that the metabolites the bacteria face are toxic to the *Leishmania* parasite, or perhaps something the bacteria generate is essential to the parasite. All of these are open questions. It was noted that those bacteria that remain modulate or provide something to the parasite to develop, and it would be interesting to address the issue, because interventions could use that approach to work against the bacteria. For example, paratransgenesis could be used to create bacteria that kill the parasite.

Participants also asked about: the maximum number of gonotrophic cycles a sand fly can complete during its lifetime; how much is known about the difference between leptomonads and retroleptomonads; and whether haptomonads ever get transmitted to the vertebrate host or whether their role is solely to modify the stomodeal valve and promote transmission of metacyclics. It was noted that Serafim's data suggest that decreasing the lifespan of sand flies in the field could reduce the possibility of transmission occurring; she asked what is known about the lifespan of sand flies in natural situations.

In response to questions about the lifecycle of the sand fly in the field, Dr. Serafim said data are limited, but there were some experiments in the early 2000s in France involving release and recapture of marked flies. Those investigators found three different blood meals in flies that were recaptured. The findings suggest that, for at least 21 days, recaptured flies have gone through several gonadotrophic cycles. It was noted that other researchers have found that when they catch infected flies, the flies had parasites from the proboscis through to the midgut. So, the amplification cycle is important to wild, infected sand flies, and the process has not been well replicated in the laboratory.

Participants asked whether the amplification in the number of parasites translated into any evidence of increased infectivity of the cells. Dr. Serafim replied that it might be a matter of probability. When his laboratory did transmission experiments, researchers found a significant increase in the number of lesions, but when the lesions appear—and they are very diminished in number among the group that did not take multiple blood meals—the lesion severity was the same. Thus, the data so far suggest that the number of lesions does not affect the virulence but rather the ability to transmit. Single-cell sequencing has not yet been conducted.

Participants commented on the need for community-wide collaboration for large-scale phenotypic screening. The NIAID-funded Eukaryotic Pathogen, Vector, and Host Informatics

Resource (VEuPathDB) supports genome-wide phenotypic data for a variety of assays (e.g., fitness, imaging, and curated phenotypes), generated using various approaches (e.g., targeted knockouts, transposon mutants, RNAi libraries, and CRISPR knockouts) for a variety of species (*Aspergillus*, *Cryptococcus*, *Neurospora*, *Plasmodium*, and *Toxoplasma*, with *Leishmania* under discussion).

Session 2: Approaches to Target the Parasite within the Vector

Each speaker gave a brief presentation focusing either on the vector or the parasite. Following presentations, participants posed questions for discussion directly or via the online chat feature.

Exposure to Antiparasitics: Ingestion Route

Parasite Speaker: Paul Bates, Lancaster University

Efforts to develop antiparasitics have focused on administering a drug to the vector to prevent transmission of the parasite to a human host, which poses challenges different from those of administering a drug to humans. The success of a transmission-blocking vaccine for the vector relies on understanding the immune response of the vector.

One route of administration for antiparasitics could be ingestion during the blood meal. For trypanosomes, the blood meal is the only ingestion route; female mosquitoes and sand flies also take sugar meals from plants. The lifecycle of the parasite is critical to the success of the ingestion route. Malaria parasites, for example, exit the gut quickly, so the window of opportunity is limited, but they could still be targeted in the gut if the antiparasitic agent could penetrate through the gut wall. To develop an antiparasitic, researchers would still have to identify the precise location to target and take into account how the parasite changes during development.

Drugs or vaccines intended to target the pathogen inside the vector will likely require ingestion of the antipathogen compound or antibodies during blood feeding on a human host. Because this process would involve the human host being bitten before the drug or vaccine has an effect on the pathogen, the drugs or vaccines would likely not have an immediate benefit for the individual; rather they would eventually benefit the community by decreasing disease transmission.

There is some proof of principle for transmission-blocking drugs targeting *Plasmodium*, and some research on transmission-blocking vaccines for *Plasmodium*. Other targets have been considered but pose significant challenges.

Vector Speaker: Marcelo Ramalho-Ortigão, Uniformed Services University of the Health Sciences

The lifecycle of *Leishmania* in the sand fly offers some potential targets for antiparasitics, particularly in the midgut. Dr. Ramalho-Ortigão demonstrated disruption in the peritrophic matrix kinetics among female sand flies that were fed anti-PpChit1 (anti-chitinase) and anti-PpPer2 (anti-peritrophin). RNAi targeting PpChit1 secreted in the midgut also reduced development of *Leishmania major* in the sand fly.

As Dr. Serafim and others have demonstrated, the status of the vector microbiome is critical to survival of the parasite. Bacteria in the sand fly larval gut colonize differently depending on the pH. Dysregulation (or dysbiosis) of the gut homeostasis leads to bacterial stimulation of the host immune response. Therefore, the microbiome could be a platform for paratransgenesis targeting either virus or bacteria. There is a great deal of conservancy between genera and species that could be exploited for delivering molecules. Technology exists to track the distribution of an element added to a sugar meal as it migrates through the sand fly.

A combined strategy of killing larvae and adults (including bacterial-induced larval killing and sugar baits) is more likely to be effective than any single approach. Local communities must be part of the decision-making process about vector and parasite control.

Discussion
It was asked whether *Leishmania* infection influences the distribution of different bacteria species in the gut. Dr. Ramalho-Ortigão replied that the infection changes the richness and diversity, but it is not yet known how it affects areas of the gut.

Participants also asked how accessible the parasites inside the matrix are to compounds and whether there are limitations in molecule size and flux across the matrix. Dr. Ramalho-Ortigão said the peritrophic matrix is fairly porous and many compounds will traverse it with ease. For example, in an experiment to assess the porosity, 75 kilodalton (kDa) to 100 kDa beads seemed to pass easily.

Exposure to Antiparasitics: Non-Ingestion Route
Vector Speaker: Flaminia Catteruccia, Harvard University
Antiparasitics can be applied to mosquito nets, circumventing the emergence of insecticide resistance. In low concentrations, atovaquone applied on a surface where a mosquito lands, for example, causes the destruction of parasites in the mosquito gut but does not affect mosquito survival or reproduction. Thus, there is little selective pressure that could lead to mosquito resistance to atovaquone. Research demonstrates that atovaquone exposure kills parasites in field- and laboratory-raised mosquitoes, even those highly resistant to insecticides.

Different drugs are needed to treat the parasites in humans and mosquitoes to mitigate the potential development of resistance to drugs delivered by mosquito nets and preserve the effectiveness of drugs used in humans. Modeling is needed to assess interventions. Novel technology can screen libraries of existing compounds for potential new drugs. New *in vitro* systems are needed to test them.

Parasite Speaker: Corey Hopkins, University of Nebraska Medical Center
Using high-throughput screening, Dr. Hopkins and colleagues identified active compounds and manipulated them to ensure they were systemically active as mosquito insecticides. The following issues should be considered in efforts to develop systemically active compounds:

- Repurposing certain human drugs may not be effective because compounds active in mosquitoes tend to have different fundamental properties (e.g., drugs for mosquitoes must be more lipophilic).

- The cost of goods is a major concern, as the compound might be used in large quantities (such as a spray for nets), which again could limit the utility of expensive human drugs.
- How the compound will reach the parasite must be considered, particularly whether through topical application or ingestion.
- Investigators should consider how to ensure the parasite receives a sufficient dose of the compound; insecticide research might offer some insights.
- Developing different drugs for humans and mosquitoes that kill the same parasites is a valuable strategy to prevent the development of resistance.

Dr. Hopkins emphasized that more cross-disciplinary collaboration is needed to identify new targets.

Discussion
Participants noted that Dr. Catteruccia's proof of principle opens the possibility of delivering antiparasitic agents through contact. It was asked whether she had thought about delivery systems and how to reach natural populations at scale. Dr. Catteruccia responded that the plan is to use bed nets or indoor spraying to deliver the antiparasitic compounds, with mosquitoes absorbing them the way they generally absorb insecticides. Therefore, the compounds must penetrate the insect cuticle.

Participants also asked how atovaquone enters the mosquito and how stable atovaquone is on surfaces over time. Dr. Catteruccia said atovaquone enters the mosquito via the tarsi, but her group is not suggesting using atovaquone on nets because it is used in humans. Stability has not yet been measured; such studies would make more sense with candidate antimalarials rather than with proof-of-principle compounds.

Finally, it was asked whether sugar baits would provide a better approach for breaking through insecticide resistance. Dr. Catteruccia said her colleagues have tested delivering antimalarials via sugar solutions and it works very well, so it may be a viable option.

Transgenics
Vector Speaker: Tony James, University of California, Irvine
Transgenesis in mosquitoes has been achieved using non-homologous recombination, homologous recombination (CAS9 and guide RNA [gRNA]), transposons, symbiotic organisms, episomes, and viruses. In mosquitoes and parasites, these techniques have been delivered by microinjection, electroporation, lipofection, and biolistics. To modulate gene expression profiles, investigators have ablated genes; altered transcription product abundance profiles; and altered tissue-, sex-, and stage-specificity. Other work has defined functional fragments of gene control sequences, introduced exogenous DNA-encoding products, and assessed enhancer traps to identify endogenous genes with specific characteristics.

Dr. James described the mechanisms by which transgenesis works in mosquitoes using class II transposable DNA elements, which integrate a fragment of DNA into a target site in the genome. The transposase is modified by donor and helper plasmids that contribute to stability. The process results in two copies of the transposable element and was originally thought to be a

potential basis for gene drives. Four transposons are frequently used for mosquitoes, and they are small but abundant enough to enable random integration.

CAS9 and gRNA systems for transgenesis in mosquitoes can target specific integration sites. The process involves homologous recombination that allows for integration into the chromosomes and is the current bases for gene drives.

Parasite Speaker: David Serre, University of Maryland, Baltimore
Investigators have applied transgenesis to insert anti-*Plasmodium* effector molecules that block *Plasmodium*, decreasing the prevalence of the parasite and the intensity of the infection. The parasite response to transgenes must be further explored to avoid potential development of resistance. Some parasites survive the anti-*Plasmodium* molecules, raising questions about how they persist and whether they remain infectious. scRNA-seq has been used to examine and compare the genetic expression profiles of parasites throughout their development in the mosquito. Current studies highlight the heterogeneity among parasites at the same anatomical location, which might explain variability in infection intensity and shed light on parasite responses to transgenes.

Improved understanding of the parasite's response can guide selection of the most promising transgenes; enable optimization by identifying when and where the effector should be expressed; and reveal ways to combine transgenes to increase their effect. Evaluating resistance in the laboratory and the molecular pathways underlying it can provide information about the likelihood of resistance emerging, especially in natural populations. With the extensive characterization of genetic diversity in multiple *Plasmodium* populations, investigators can identify populations most susceptible to developing resistance. Studying resistance in the laboratory could identify biomarkers associated with resistance before transgenes are deployed in the field.

Among the challenges ahead are determining whether a safe, effective, and sustainable genetic vector control can be developed, as well as what constitutes effectiveness. Investigators need living strain resource repositories to support research. Some opportunities in the field are as follows:

- Probe mosquito/parasite interactions for vulnerabilities amenable to genetic targeting.
- Develop surrogate assays to validate *P. falciparum*-resistant mosquitoes.
- Develop and test genetic mitigation strategies in anopheline mosquitoes.

Microbiome/Paratransgenesis

Vector Speaker: Rita Rio, West Virginia University
Advancing paratransgenesis requires understanding the biology of the microbiota. Within parasite/vector systems, symbiont type, location, and mode of transmission vary. Although genetic tools for assessment are readily available to culture and transform *Sodalis glossinidius* bacteria (a symbiont of the tsetse fly), for example, few exist for other symbionts. Another tsetse fly symbiont, *Wigglesworthia glossinidia*, is pivotal to tsetse fly nutrition and immunity, most notably providing B vitamins that are important for reproduction, development, and vector competence. *W. glossinidia* also plays a crucial role in immunity.

Sodalis precaptivus is a novel candidate for paratransgenesis because it is less susceptible to genome degradation than *S. glossinidius*. When introduced in tsetse flies, it has no effect on the fly's lifespan but does affect the reproductive tissue, resulting in vertical transmission in a single generation that continues for subsequent generations.

Obligate microbes, if available, have high host specificity and transmissibility; they could be used as targets or vehicles for paratransgenesis. Some other microbes may be useful for paratransgenesis, such as *S. glossinidius*, which is easy to manipulate using existing tools, or *S. precaptivus*, and lessons learned may be applicable to other insect systems. The effector modules may or may not have broad-spectrum effects.

Bacteria Speaker: Laura Runyen-Janecky, University of Richmond
Paratransgenesis can target the vector (e.g., with nanobodies that target parasite surface proteins essential for development), the parasite (e.g., with bacteria that express antiparasitic proteins or molecules), or the symbiont. Because *S. glossinidius* is related to *Escherichia coli*, investigators have adapted some of the many genetic tools available for *E. coli* to develop a robust genetic toolkit for *S. glossinidius* that enables horizontal gene transfer, chromosome alteration, and transgene expression and product secretion.

For the trypanosome/tsetse dyad, much is known about biological considerations, but investigators have yet to understand the implications of replacing a native insect population with a paratransgenic population and the fitness costs to symbiotic systems. Knowledge gaps persist around technical aspects. Although a robust genetic tool kit is available for *S. glossinidius*, such resources are lacking for *W. glossinidia* and other bacteria. The field would benefit from the ability to identify more transgene effectors and their specificity. The field also needs a set of motor and secretion signals to enable investigators to control when, where, and how transgenesis occurs so they can fine-tune paratransgenesis. Implementation of any strategy requires understanding how it integrates with other control strategies, as well as recognition of the social, environmental, ethical, and regulatory issues, which requires conversations with all stakeholders.

Discussion
Participants asked what is known about the fitness impact for the insects, native microbiota, or pathogens. Dr. Runyen-Janecky said that elimination of *Wigglesworthia* has a huge effect on the fitness of tsetse because *Wigglesworthia* provides essential B vitamins to tsetse that are missing from the fly's vertebrate blood-specific diet and that facilitate larval developmental pathways essential for immunocompetency during adulthood. Additionally, *Wigglesworthia* provides thiamine to *Sodalis*, which is important because *Sodalis* is a thiamine auxotroph. Less is known about the effect of *Sodalis* on tsetse fitness. Elimination of *Sodalis* has been shown to reduce tsetse lifespan. There is a correlation between *Sodalis* density and trypanosome infection prevalence in tsetse; specifically, high densities of *Sodalis* in the tsetse midgut are correlated with the tsetse's significantly increased susceptibility to infection with trypanosomes. The mechanism that underlies this finding is not yet elucidated.

It was asked which tissues of tsetse *S. precaptivus* colonize in the transformed tsetse. Dr. Rio responded that the gut and reproductive tissue of males and females are infected with *S. precaptivus*, and these infections persist throughout the tsetse lifespan.

Participants noted that, with respect to malaria parasite development in the mosquito vector and the microbiome, colleagues at the biotechnology company Sanaria use aseptic mosquitoes for *P. falciparum* sporozoite production, and there appears to be no requirement for additional microbes for their development.

Session 3: Tools to Accelerate Research

Introduction to Session 3

Steve Tsang, Office of Data Science and Emerging Technologies (ODSET), NIAID
Computational and informatics research at NIAID is driven by basic and clinical scientific research needs; they generate large, diverse data sets and novel technological solutions. Genomic resources, systems biology, and modeling can be used to accelerate development and discovery in vector biology and parasitology.

Tools for Managing, Integrating, and Mining Complex Datasets

Eukaryotic Pathogen, Vector, and Host Informatics Resource (VEuPathDB) - Mary Ann McDowell, University of Notre Dame
VEuPathDB (https://veupathdb.org) is a NIAID-funded Bioinformatics Resource Center that offers data along with resources to use those data. More than 230 species are supported, and the database facilitates queries about orthological relationships. Eventually, VEuPathDB will merge with the Clinical Epidemiology Resources Database (ClinEpiDB, https://clinepidb.org/ce/app/) and the Microbiome Database (Microbiome DB, https://microbiomedb.org/mbio/app). It has 1,700 datasets; new datasets and new tools are added every few months. Genome sequence and annotation data, phenotypes, field and clinical data, and numerous other data types are supported.

VEuPathDB provides a range of analytical tools that can be applied to the supported datasets or to one's own data. Tools include Apollo, BLAST, and MapVEu, which supports global data analysis. Dr. McDowell walked through some of the search capacities and filtering systems available to help users of VEuPathDB find the information they want. In addition to online tutorials and educational materials, webinars, and workshops, VEuPathDB hosts virtual trainings for laboratories on request. Investigators are encouraged to contact VEuPathDB with questions, suggestions, and proposals for new datasets to add (https://veupathdb.org/veupathdb/app/contact-us).

Clinical Epidemiology Database (ClinEpiDB) - David Roos, University of Pennsylvania
NIAID's Vaccine Resource Center ensures that a wealth of genomic and transcriptomic information is accessible for discovery and translation and provides tools for identifying and comparing data. Data can be mined to identify genes of interest and to conduct *in silico* experiments. To address the difficulty of comparing disparate epidemiologic datasets, NIAID has embarked on semantic harmonization efforts, motivated, for example, by the work of the International Centers for Excellence in Malaria Research to establish common definitions.

ClinEpiDB leverages the infrastructure and tools created for VEuPathDB to facilitate access, comparison, and mining of epidemiological data sets. Users can craft searches and analyze variables to assist with investigations and even generate new hypotheses.

Discussion
Participants noted that many vectors also transmit viruses and asked how such datasets could be linked. Dr. McDowell said she is working closely with VEuPathDB's sister entity, the NIAID-funded Bacterial and Viral Bioinformatics Resource Center (https://www.bv-brc.org/), to identify use cases and combine resources.

It was asked whether at some point vector microbiota or endosymbiont genomes may be linked. Dr. McDowell said the first mosquito microbiome dataset would be released shortly, and there is interest to host additional datasets on MicrobiomeDB. Dr. McDowell added that the power of the three NIAID-funded databases lies in the harmonization of ontologies and the common underlying infrastructure, which makes data interoperable across the three platforms.

Participants asked whether a similar resource is available for ticks and sand flies. Dr. Roos responded that both are included in VectorBase (https://vectorbase.org/vectorbase/app) and he added that *Leishmania* genomics data are supported by TriTrypDB (https://tritrypdb.org/tritrypdb/app) both databases are components of VEuPathDB). A leishmaniasis skin microbiome dataset is available in MicrobiomeDB, but no leishmaniasis data are yet available in ClinEpiDB. Dr. Roos said the platform is very well suited to the complex interplay between host/vector/pathogen genetics, biochemistry, and immunology, so, is keen to talk with those who may have field or clinical leishmaniasis datasets appropriate for ClinEpiDB. Roos noted that he would also be happy to discuss adding tick microbiome data to MicrobiomeDB and field study data to ClinEpiDB.

There was a discussion about the data, with participants pointing out that that the data, especially from population studies, are very heterogeneous, and it could be a mistake to try to harmonize them. The most an ontology can hope to do is aggregate the data and draw attention to the differences in definitions, study designs, and other features. Dr. McDowell said that, for the population data, harmonization focuses mostly on enabling users to identify data. She agreed that it can be difficult to harmonize different population studies but noted that individuals can explore several studies to generate new hypotheses to investigate in a controlled manner. Dr. Roos added that ClinEpiDB does not support queries across studies because of the difficulty of harmonizing data from complicated datasets from the field. He said that ontologies help to highlight what data are available in which studies and enable similar queries against similar datasets but does not wish to foster a false sense of confidence by integrating data that cannot be combined.

Systems Biology Approaches to Manage the Complexity of Parasite/Vector Interactions
Need for a Systems Biology Approach - Shirley Luckhart, University of Idaho
Systems biology is needed to understand the complex interface across vectors, pathogens, and hosts, with particular attention to the crosstalk between metabolism and the regulation of pathogen transmission in arthropods. Arthropods rely on a number of signaling pathways that are highly interconnected and together regulate metabolic processes that define resistance, behavior, longevity, reproduction, and immunity. Basal and modified states of host metabolism define the outcomes of infection and manipulations to enhance host defenses. Research should look at variation over time within the individual, within populations, and across populations—for hosts and vectors.

Dr. Luckhart offered an example of how vitamin B5, needed to synthesize coenzyme A, can be manipulated to elucidate how variation affects mosquitoes at the individual level, which might be an approach to controlling malaria parasite infection within the mosquito. Given the complexity of pathogen development in arthropod vectors, researchers can leverage systems biology to identify the master regulators in parasite/vector interactions and guide experimental design to examine the largest proportion of phenotypic variation in pathogen development.

Combining Information for a Systems Biology Approach – Adam-Nicolas Pelletier, RPM Bioinfo Solutions and Emory University
Systems biology allows investigators to generate novel insights by combining information from various "omics" to build powerful models that can be adapted to the parasite/vector interface. The limited amount of biological material is a challenge in arthropod research, so tissue is often combined—at the expense of understanding individual variation. Moreover, protocols for the isolation of analytes such as metabolites have proven difficult in the past. Spatial transcriptomics captures biological heterogeneity in situ, providing context-dependent information that even scRNA-seq cannot. This approach can, for example, illustrate at the genetic level where parasites are located, what they express, their developmental stages, and their interaction with the vector.

Other spatial technologies are being developed, such as spatial metabolomics. For example, a technique combining fluorescence in situ hybridization with mass spectrometry captures molecules and bacterial symbionts in fish. Combining technologies could enable investigators to generate models of the parasite/vector interface.

Discussion
It was asked what factors are limiting the field from applying the spatial omics technologies to vector research. Dr. Luckhart said research has been impeded by the spatial scale and the amount of material available. High costs have been a barrier to accessibility, although that is not unique to vector biology. There has long been discussion about establishing national resource centers to promote sharing of technology that allows researchers to conduct the preliminary work needed for grant applications and data publication. Dr. Luckhart hoped this workshop would spur more conversation on the idea.

Modeling Parasite/Vector Interactions to Assist in Target Identification

Model Characteristics and Considerations - David Smith, University of Washington
Modeling turns ideas into rigorous equations that describe the processes involved. Models can take a statistical approach—identifying patterns based on data, which looks at effects to determine potential causes—or a mechanistic approach, in which the cause is presumed, and the model demonstrates the effects. Dr. Smith advocated for combining both approaches to close the loop, building models for prediction and for testing the conclusions in a broader context. In addition, models must take scale into account, recognizing that the processes at one scale create effects at another scale. Models for scientific inference identify and codify what is known; models for analytics assist with decision making; the differences between the two lie in how each deals with uncertainty. The quality of a model can be judged by what the model is intended to do, but some key factors include accuracy, utility, and appropriate incorporation of data.

Malaria is heavily influenced by local conditions, so it is not clear that what works in one area will work in another. Researchers need to understand what does or does not work before they can determine whether an approach translates effectively. Smith outlined a model for adaptive malaria control that involves an iterative, cyclical process of gathering data to inform action.

An Environmental Model of Leishmaniasis - Caroline Glidden, Stanford University
Visceral leishmaniasis has been spreading across Brazil for two decades. *Leishmania infantum* is typically transmitted from flies to dogs. When a host of environmental factors align, the pathogen spills over to humans. But the processes are not linear; they occur on multiple scales, and a great deal of data are missing. For example, host and vector distribution are affected by land use and climate, and vector competence is influenced by temperature. Dr. Glidden used machine learning to create models that use environmental data to fill in missing data and evaluate which environmental variants are most useful for predicting spillover across different land environments. The model performs well and has high specificity. Notably, the most predictive variables are land type and temperature.

The model was intended to generate hypotheses, which are now being tested via a mechanistic model. For example, the first model identified that sand flies become infectious after feeding in a given air temperature, and the second model will estimate infections by air temperature over time. Once the model is created, other environmental variables can be incorporated to enhance the ability to predict changes in disease spread according to land type and use and climate change.

Discussion
Participants appreciated the challenge of determining what kinds of observations are transferable. It was asked what datasets were used for training the machine learning model assessing leishmaniasis in Brazil and how accurate the model has been at predicting spillover when applied retroactively. Dr. Beverley noted that data on leishmaniasis come from clinical cases and represent just the tip of the iceberg of *Leishmania* infections; some studies indicate that infections are 10-fold to 50-fold higher than cases. Dr. Beverley also pointed out that, increasingly, many mammals other than dogs are being implicated as *Leishmania* hosts.

Dr. Glidden responded that she has been using ERA5 climate data, MapBiomas land-use data, and United Nations population data. So far, these sources have been quite accurate at retroactively predicting pathogen spillover. The out-of-sample area under the receiving operator characteristics curve is on average 0.86 (model trained with 80% of data and validated with 20% of data). Dr. Glidden added that she is also developing a model that uses machine learning to identify reservoir hosts and vectors and then will evaluate their contribution to transmission via the spillover model.

It was noted that data will rarely identify more than the tip of an iceberg. Models can assist with synthesis, which generally helps identify critical knowledge and data gaps and quantify uncertainty. Participants said Dr. Glidden's studies are an outstanding example of how to make the most of existing cheap and available data. Dr. Beverley clarified that the model maps disease,

rather than parasites, which is not a problem if the relationship between infection and disease is constant, but it may not be.

Informatics Research at NIAID: Opportunities and Challenges in Vector-Borne Disease Research - *Steve Tsang, ODSET, NIAID*

ODSET builds partnerships across NIAID to harness the power of data for research. As more data are generated, enabling discovery involves encouraging investigators to use and reuse the valuable data collected. ODSET aims to make research more findable, accessible, interpretable, and reusable—or FAIR—and more robust.

A number of informatics funding opportunities are available (https://www.niaid.nih.gov/research/data-science-funding-opportunities). Three trans-NIAID technology development funding opportunities support exploratory data science methods and algorithm development (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-21-035.html); early-stage development of data science technologies (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-21-020.html), and enhancement or sustainment of data science tools for infectious and immune-mediated diseases (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-21-021.html). For data reuse, a notice of special interest is available to support secondary analysis of existing datasets for advancing immune-mediated and infectious disease research (https://grants.nih.gov/grants/guide/notice-files/NOT-AI-21-011.html).

For workforce development, NIAID and the National Heart, Lung, and Blood Institute offer a summer institute for research education in biostatistics and data science to complement and enhance training (https://grants.nih.gov/grants/guide/rfa-files/RFA-HL-22-009.html). A Small Business Innovation Research contract supports the development of informatics tools (https://www.niaid.nih.gov/grants-contracts/2021-sbir-contract-solicitation). The National Science Foundation's Smart and Connected Health vehicle supports research involving artificial intelligence and advanced data science (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-21-011.html).

With more projects generating large, diverse, complex data sets, NIH and NIAID recognize the need for data that align with the FAIR principles and for innovative, sustainable technology solutions. NIAID encourages repositories to follow the principles of transparent, responsible, user-focused, sustainable technology—or TRUST—as a framework to implement best practices and enhance digital preservation by all stakeholders. Finally, there is a critical need to transform data into knowledge by accelerating research in many areas.

Discussion: Leveraging Tools for Research

Participants pointed out that a tool is only as good as the data it incorporates and asked how to assess the quality of data sets. Dr. Tsang replied that ODSET seeks to raise awareness about NIH policies and best practices for data management and sharing, which support quality control.

It was also asked how ODSET is working with investigators to help improve interoperability across systems, such as supporting curation within grantee institutions. Dr. Tsang responded that ODSET works with NIAID program officers to help them understand NIH policies.

Participants discussed the U.S. government's research funding approach and difficulties in obtaining population-level information. It was asked how to get funding to support understanding of the vector ecology for diseases. Dr. Tsang responded that the trans-NIAID technology development funding calls for a computational approach but leaves the scientific question open, so applicants could propose a mechanism for vector ecology.

Participants observed that it is difficult to create multiscale models that combine ecological and organism data and allow the user to toggle between infectious disease epidemiology and ecology. They noted that NIAID funding opportunities are nicely attuned for certain research aspects, but there may be a gap in implementation.

Dr. Ramalho-Ortigão said that NIH has partnered with the U.S. Department of Defense (DoD) for various areas of research, and such collaborations could be used for population and ecology research in the future.

Participants pointed out that although there is broad support for the concept of FAIR data access, the easier it is to *reuse* data, the easier it is to *misuse* data. Minimizing the potential for inadvertent misinterpretation is a major preoccupation for all the data-mining resources. Dr. Smith responded that he believes it is a mistake to try to regulate misuse of data; rather, resources should highlight the heterogeneity of the data.

It was emphasized that there is a need for more funding for work at the organism and population levels. Dr. McDowell pointed to the emerging infectious diseases program supported by the National Science Foundation and NIAID. Participants noted it was highly focused on basic rather than applied research.

Finally, it was noted that vector ecology is a neglected issue. Dr. Roos added that disease ecology in general is neglected.

Reports from the Breakout Groups

On day two, participants met in groups for approximately 90 minutes to identify limitations, opportunities, mechanisms for enhancing target discovery, and research priorities from the research community perspective for five parasite/vector pairs.

Mosquito-Borne Diseases

Limitations

- Little is known about mosquito/pathogen interactions (e.g., what the pathogen requires for development).
- Performing mosquito infections is difficult and requires expertise in rearing and an insectary.
- Transgenic and field-derived mosquitoes cannot be cryopreserved.
- There is limited access to field or semi-field sites to perform assays and studies.

Opportunities

- Develop *in vivo* and *in vitro* models to study pathogen/mosquito interactions outside of an insectary.

- Establish a central facility for mosquito storage and preservation.
- Develop standardized protocols for assays.
- Develop simplified procedures for transgenesis.
- Create resources to facilitate interaction and collaboration within and across fields (e.g., connect basic scientists with modelers).

Mechanisms for Enhancing Target Discovery

- Development of *in vivo* and *in vitro* models and tissue systems to study pathogen/mosquito interaction.
- Use of bioengineered tissues or 3D-printed tissue platforms.

Identified Research Priority Areas

- Develop *in vivo* and *in vitro* models to study pathogen/mosquito interaction.
- Develop ability and facility for long-term storage of transgenic and field mosquitoes.

Sand Fly-Borne Diseases

Limitations

- There is a lack of reliable field studies to understand sand fly ecology and epidemiology.
- Genetic tools for sand flies are limited.
- Sand fly colonies are difficult to rear and maintain, and few laboratories do so.
- There is limited access to animal facilities and the regulatory requirements of keeping animals pose barriers.
- There is no robust regulatable genetic system for *Leishmania*.
- The field lacks standardization of culturing *Leishmania* lifecycle stages.
- Shipping materials is difficult.

Opportunities

- Use transposons (e.g., piggyBac) for genetic manipulation.
- Provide training and travel grants for investigators to learn how to maintain sand fly colonies.
- Apple single-cell sequencing, especially in situ, to better understand the lifecycle of *Leishmania* in the midgut, for example.
- Discover a lifecycle regulatory switch for *Leishmania*.
- Conduct microbiome studies, especially in the field.
- Use antiparasitics in the vector.
- Use the NIAID-funded BEI Resources Repository for distributing material (https://www.niaid.nih.gov/research/bei-resources-repository).
- Use the Drugs for Neglected Diseases Initiative for drug development (https://dndi.org).
- Apply a One Health approach.

Mechanisms for Enhancing Target Discovery

- Effective genetic tools for research on sand flies.
- Comparative approaches leveraging information from other organism systems (e.g., *Drosophila*, mosquitoes, trypanosomes).
- Enhanced modeling and genomics training for *Leishmania* and sand fly researchers.

Identified Research Priority Areas

- Enhance understanding of sand fly ecology.
- Develop a regulatable genetic system for *Leishmania*.
- Enhance modeling approaches for control interventions.

Tsetse Fly-Borne Diseases

Limitations

- The lack of access to tsetse flies is the main bottleneck for the research community.
- The United States lacks fly-transmissible RNAi lines for loss-of-function experiments.
- It is not clear whether the fly and parasite lines available reflect what is in the field in terms of microbiota and vector competence.
- There is a lot of misinformation around the annotation of genomic resources.
- The lack of vertical transmission of the genetically modified microbiome for paratransgenesis poses a barrier.
- Chemotactic and developmental signals in the fly host that enable parasite transmission are unknown.
- There are limited metabolomic, glycomic, and spatial-omic investigations.

Opportunities

- A vertically transmitted, ancestral symbiont has been identified. Consider using it for paratransgenic lines and modifying tsetse gene expression.
- Improve annotation through computational approaches.
- Take advantage of field insectaries available in endemic countries.
- Expand the research community (e.g., to include Europe and Africa).
- Apply new imaging approaches to research.

Mechanisms for Enhancing Target Discovery

- Whole-genome parasite RNAi in the fly (e.g., RNAi target sequencing) for functional genomics.
- RNAi parasite lines that are transmissible in the fly.
- Genome curation for parasite and vector.
- Parasite development in the fly, e.g., lightsheet microscopy, focused ion beam scanning electron microscopy, and serial block-face scanning electron microscopy.
- Symbiont cultures that are cultivatable, modifiable, and vertically transmissible.
- Understanding of the role of *Wigglesworthia* symbiosis in parasitism.

Identified Research Priority Areas

- Expand biological resources (e.g., parasite lines, symbiotic lines).
- Expand infrastructure for tsetse resources (for laboratory and field colonies).
- Expand computational resources (e.g., imaging, curation of data).
- Conduct functional genomic investigations during transmission cycle (parasite/fly interactions).

Triatomine-Borne Diseases

Limitations

- The field lacks genetic tools for the vector and the parasite.
- There is little understanding about parasite diversity and vector specificity for discrete typing units or genetic lineages.
- The field lacks omics data (especially for the vector).
- Availability of insect colonies is limited; maintenance is expensive, labor-intensive, and limited to a few species.
- There is little communication between parasite and vector researchers.

Opportunities

- Apply or adapt tools from other systems (e.g., inducible systems to alter gene expression in the host and CRISPR in the vector).
- Employ *in vivo* transcriptomics and scRNA.
- Organize more scientific meetings for the community.
- Explore the paratransgenic approach.

Mechanisms for Enhancing Target Discovery

- Drug screening in the vector.
- Identification of vector and parasite targets.
- Robust regulatory manipulation of both organisms.

Research Priorities

- Fill the holes in the basic biology (high priority).
- Apply tools from other systems.
- Increase communication within the community.
- Unify methods for strains and vectors.
- Address the outstanding needs to enable progression to translational studies.

Tick-Borne Diseases

Limitations

- There is a knowledge gap around emerging and introduced species of ticks and tick-borne diseases.
- There is poor understanding of the ecology of ticks and tick-borne pathogens.
- Little is known about pathogen/vector interactions.
- Validation of laboratory models compared with natural systems is needed.
- There are few consolidated resources to support genomics and transcriptomics.
- Functional genomics resources and tools are needed:
    - Genomes
    - Computational tools
    - CRISPR
    - Computing power and cloud resources
- Little research is performed in resource-limited settings (e.g., Africa, Asia, and Central and South America).

Opportunities

- For emerging and introduced species of ticks, evaluate the following:
    - Vector/pathogen/host competence
    - Ecology
- Distribution and range of ticks and tick-borne diseases is increasing. Evaluate the following:
    - Ecology
    - Vector/host competence
    - Coinfections
- Increase research on the following tick-borne pathogens:
    - *Babesia* species
    - Powassan or deer tick, Heartland, and other viruses
    - Spotted fever group *Rickettsia*
    - *Borrelia* species
- Develop a consortia for sharing *in vitro* and *in vivo* resources.
- Explore molecular tools for vector and pathogen manipulation.

Mechanisms for Enhancing Target Discovery

- Cell lines for target identification of non-model vectors.
- Identification of markers to target the vector on the vertebrate host (e.g., for anti-vector-based vaccines).
- Identification of markers to target the pathogen in the vector or anti-pathogen vaccine.

Research Priorities

- Develop infrastructure and consortia for ticks and tick-borne pathogens.
- Develop molecular tools and in vitro resources for vector manipulation.
- Establish infrastructure for data analysis and biostatistics for large data sets.
- Conduct focused research on ecological aspects and vector competence of understudied, emerging, and introduced tick species and pathogens.

Cross-Cutting Themes - *Mike O'Neill, DMID, NIAID*

From the discussions and breakout team presentations, several cross-cutting themes and needs were identified, including the following examples:

- Core facilities are needed to generate reagents and organisms (pathogens and vectors) to increase consistency and reproducibility.
- The field of vector/pathogen interactions needs new ideas from a broader audience with different perspectives. A web-based platform for linking investigators across research specialties might be a good start to enable multidisciplinary collaborations and facilitate the exchange of ideas.
- Better information and data management and sharing are needed across research fields.

Final Remarks - ***Adriana Costero-Saint Denis, DMID, NIAID***

The communication platform established for this workshop could serve as vehicle for sharing information and promoting collaboration. Participants are encouraged to use it to keep in touch with each other and to provide feedback to NIAID. The value of the platform depends on how the research community uses it. To join the platform or provide feedback on the workshop, please email Dr. Adriana Costero-Saint Denis at acostero@niaid.nih.gov.

Appendix A: Participants List

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Appendix B: Resources and Background Materials

Investigator Resources

- NIAID's BEI Resources for vector research (https://www.beiresources.org/Home.aspx) suggested search terms:
    - Interventional agent development services
      Preclinical models of infectious diseases
      Therapeutic development services
    - In vitro assessment for antimicrobial activity program
    - Vaccine development services

- NIAID's Eukaryotic Pathogen, Vector, and Host Informatics Resource (VEuPathDB)
    - Clinical Epidemiology Resources Database (ClinEpiDB)
    - Microbiome Database (Microbiome DB)
    - VectorBase
    - Kinetoplastids Informatic Resource (TriTrypDB)
- NIAID's Bacterial and Viral Bioinformatics Resource Center
- Drugs for Neglected Diseases Initiative

Funding Opportunities

- NIAID Data Science Funding Opportunities
- NIAID Funding Opportunities
- NIAID Systems Biology for Infectious Diseases: Request for Applications

NIAID Information and Contacts

- NIAID Division of Microbiology and Infectious Diseases (DMID)
- Preclinical and Clinical Services contacts
- *Within DMID*:
    - For any vector control product development and research resources or support and for Preclinical Services related questions, contact Ghiorghis Ghenbot at ghiorghis.ghenbot@nih.gov.
    - For questions regarding NIH funding mechanisms to support research on vectors and vector-borne pathogens, contact Adriana Costero-Saint Denis at acostero@niaid.nih.gov.
    - For questions regarding parasite drug discovery and development, contact Michael O'Neil at michael.o'neil@nih.gov.

Appendix C: List of Selected Key Publications

Session 1: State of Science for Parasite/Vector Systems

1. ***Trypanosome/Tsetse Fly***
    - Mammalian African trypanosome VSG coat enhances tsetse's vector competence.
    - A fine-tuned vector-parasite dialogue in tsetse's cardia determines peritrophic matrix integrity and trypanosome transmission success.
    - Developmental Progression to Infectivity in Trypanosoma brucei triggered by an RNA-Binding Protein.
    - Identification of positive and negative regulators in the stepwise developmental progression towards infectivity in Trypanosoma brucei.
2. ***Trypanosome/Triatomine Bug***
    - From e-voucher to genomic data: Preserving archive specimens as demonstrated with medically important mosquitoes (Diptera: Culicidae) and kissing bugs (Hemiptera: Reduviidae).
    - Interactions among Triatoma sanguisuga blood feeding sources, gut microbiota and Trypanosoma cruzi diversity in southern Louisiana.
3. ***Malaria/Mosquito***
    - A Plasmodium key fits a mosquito lock.
    - Mosquito Microbiota and Implications for Disease Control.
    - Type II Fatty Acid Biosynthesis Is Essential for Plasmodium falciparum Sporozoite Development in the Midgut of Anopheles Mosquitoes.
    - Driving mosquito refractoriness to Plasmodium falciparum with engineered symbiotic bacteria.
    - The Plasmodium bottleneck: malaria parasite losses in the mosquito vector.
4. ***Leishmania/Sand Fly***
    - Sequential blood meals promote Leishmania replication and reverse metacyclogenesis augmenting vector infectivity.
    - Distinct gene expression patterns in vector-residing Leishmania infantum identify parasite stage-enriched markers.
    - Heme Oxygenase-1 Induction by Blood-Feeding Arthropods Controls Skin Inflammation and Promotes Disease Tolerance.
    - Gut Microbes Egested during Bites of Infected Sand Flies Augment Severity of Leishmaniasis via Inflammasome-Derived IL-1beta.
    - Sequential blood meals promote Leishmania replication and reverse metacyclogenesis augmenting vector infectivity.
    - Leishmaniasis: The act of transmission.

Session 2: Approaches to Target the Parasite within the Vector

1. ***Exposure to Antiparasitics: Ingestion Route***
    - An integrated overview of the midgut bacterial flora composition of Phlebotomus perniciosus, a vector of zoonotic visceral leishmaniasis in the Western Mediterranean.
    - Zoonotic visceral leishmaniasis transmission: modeling, backward bifurcation, andoptimal control.
    - Bacterial Infection and Immune Responses in Lutzomyia longipalpis Sand Fly LarvaeMidgut.
    - Evaluation of two sexual-stage antigens as bivalent transmission-blocking vaccines inrodent malaria.
    - The transmission-blocking effects of antimalarial drugs revisited: fitness costs andsporontocidal effects of artesunate and sulfadoxine-pyrimethamine.
    - Transgenic pyrimethamine-resistant plasmodium falciparum reveals transmission-blocking potency of P218, a novel antifolate candidate drug.
    - Transmission blocking sugar baits for the control of Leishmania development inside sandflies using environmentally friendly beta-glycosides and their aglycones.
2. ***Exposure to Antiparasitics: Non-Ingestion Route***
    - Discovery and characterization of 2-nitro-5-(4-(phenylsulfonyl)piperazin-1-yl)-*N*- (pyridine4ylmethyl)anilines as novel inhibitors of the *Aedes aegypti* (*Ae*Kir1) channel.
    - An insecticide resistance-breaking mosquitocide targeting inward rectifier potassiumchannels in vectors of Zika virus and malaria.
    - Discovery and characterization of a potent and selective inhibitor of *Aedes aegypti* inward rectifier potassium channels.
    - Exposing Anopheles mosquitoes to antimalarials blocks Plasmodium parasitetransmission.
    - Antimalarials in mosquitoes overcome Anopheles and Plasmodium resistance to malariacontrol strategies.
3. ***Transgenics***
    - Population modification of Anopheline species to control malaria transmission.
    - Next generation gene drive for population modification of the malaria vector mosquito,Anopheles gambiae.
    - Efficient population modification gene-drive rescue system in the malaria mosquitoAnopheles stephensi.
    - Transcriptional heterogeneity and tightly regulated changes in gene expression during *Plasmodium berghei* sporozoite development.
    - Single-cell transcription analysis of *Plasmodium vivax* blood-stage parasites identifies stage- and species-specific profiles of expression.
    - Genomic Analyses Reveal the Common Occurrence and Complexity of

Plasmodium vivaxRelapses in Cambodia.

4. ***Microbiome/Paratransgenesis***
    - Delivery of a functional anti-trypanosome Nanobody in different tsetse fly tissues viaabacterial symbiont, Sodalis glossinidius.
    - Engineering insects from the endosymbiont out.
    - Genetic Modification of Sodalis Species by DNA Transduction
    - Self-limiting paratransgenesis.

Session 3: Tools to Accelerate Research

1. ***Genomic Resources***
    - ClinEpiDB: an open-access clinical epidemiology database resource encouraging onlineexploration of complex studies.
    - MicrobiomeDB: a systems biology platform for integrating, mining and analyzingmicrobiome experiments.
    - VEuPathDB
    - Advances in omics-based methods to identify novel targets for malaria and otherparasitic protozoan infections
    - The transcriptome of circulating sexually committed Plasmodium falciparum ring stageparasites forecasts malaria transmission potential
2. ***Systems Biology***
    - A systems approach to infectious disease
    - Studies of the Parasite-Midgut Interaction Reveal Plasmodium Proteins Important forMalaria Transmission to Mosquitoes
    - Mapping Arbovirus-Vector Interactions Using Systems Biology Techniques
3. ***Modeling***
    - Why Model Malaria?
    - Human mobility patterns and malaria importation on Bioko Island
    - A New Test of a Theory about Old Mosquitoes
    - Evolutionary consequences of feedbacks between within-host competition and diseasecontrol
4. ***Data Science***
    - Infectious Disease Research in the Era of Big Data
    - Artificial Intelligence for infectious disease Big Data Analytics