Results of the small-scale release of non gene drive genetically modified sterile male mosquitoes in Burkina Faso

Summary

On 1 July 2019, the Research Institute of Health Sciences (*Institut de Recherche en Sciences de la Santé*/IRSS) carried out the first release of genetically modified mosquitoes in Africa, as part of Target Malaria in Burkina Faso. This release marked an important step in the overall research pathway for developing a genetic technology to fight malaria undertaken by the Target Malaria consortium.

Originally imported from our partner, the Polo d'Innovazione di Genomica, Genetica e Biologia (Polo GGB), in Terni, Italy, in the form of eggs in 2016 under a contained use permit issued by the Burkinabe National Biosafety Authority (ANB), the colony of genetically modified mosquitoes was established and studied by the medical entomology team at the IRSS insectary in Bobo-Dioulasso. Following these studies, an application for a controlled release was prepared and submitted by IRSS and approved by the ANB.

Following the release, seven months of monitoring enabled the team to draw scientific conclusions on the survival and behaviour of the modified mosquitoes. This phase was essential for gathering information, sharing the research steps with stakeholders, working with the regulatory authorities, transferring knowledge, and developing local skills. Its objective was not to control mosquito density or impact malaria transmission.

The study built on several years of preceding entomological field work and stakeholder engagement efforts at the study sites. Beyond the research objectives and validation of study protocols, the study served to co-develop, inform and share research steps with a broad range of stakeholders from communities to national regulatory authorities, as well as transfer knowledge and build capacity between project partners.

The research, stakeholder engagement and regulatory goals for this release were achieved: establishing release protocols with the authorities, gaining the agreement of the communities and demonstrating safely how the insects behave in the field.

The non gene drive sterile male mosquitoes

This first phase in our research programme involved experimenting with a strain of non gene drive sterile male mosquitoes. When these non gene drive sterile male mosquitoes mate with females, the eggs laid by the females do not hatch. Sterility is caused by a genetic modification that only affects the generation of modified mosquitoes and cannot be passed onto the next generation because the modified insects are sterile.

This non gene drive sterile male is genetically modified but does not carry a gene drive element that would otherwise bias its inheritance. The strain was generated at Imperial College London. After molecular characterisation, the mosquitoes were tested in containment at Polo GGB.

Before the arrival of the eggs at the IRSS research laboratory, an intensive engagement with stakeholders living around the insectary - including a public consultation – was carried out by the team to ensure that the community was aware of the upcoming activities in the insectary and to seek their agreement.

Studying the sterile male mosquitoes in containment in Burkina Faso

Following the original importation of the non gene drive sterile male eggs from PoloGGB, the IRSS team repeatedly backcrossed the sterile male strain with a strain with the genetic background of local populations of mosquitoes in Burkina Faso so that the strain would be as similar as possible to it. The objective was to replace the lab-adapted genetic background with a background more representative of the local population. The independent risk assessment for this release supported this conclusion[1].

Permission for the field release

In November 2017, the IRSS submitted a regulatory application to the ANB seeking authorisation to carry out a small-scale release of the non gene drive sterile male mosquitoes. The IRSS received authorisation in August 2018 from the ANB to carry out the requested release.

Target Malaria started its engagement work in the community of Bana (the village of the release) in 2012 with a dialogue with community members about their way of making decisions for activities taking place in the common areas. The model is based on the idea that a group of representatives from the community voice the community decision, in alignment with the traditional decision-making process.

The composition of this group was made on the basis of ethnographic studies carried out in the village and with a verification with more vulnerable groups (including women, and ethnic minorities) that the members were considered legitimate.

After a public consultation by the IRSS team with community representatives on the release, the Bana community provided its agreement to IRSS in May 2018. The community decision was formalised through the signature of a form that was complemented by an information sheet and also approved by the IRSS Institutional Ethics Committee. This model is aligned with the existing governance structures of the community and therefore respects their tradition and culture. It was also cross-checked against authoritative guidelines for responsible research in this field (WHO 2014, NASEM, 2016).

Community-based grievance management and monitoring committees were also established to receive and give feedback on the release and to enhance the community's involvement prior to, during and after the release. In addition to this formal consultation with the group of representatives, the project carried out many meetings and consultations prior to the release to ensure that the community felt informed enough and that there was broad agreement for the release.

The release on 1 July 2019

Following regulatory authorisation, the IRSS team carried out the small-scale release of non gene drive sterile male mosquitoes, in the village of Bana in the district 7 of Bobo-Dioulasso on 1 July 2019.

Approximately 6,400 genetically modified male mosquitoes and approximately 8,500 non-genetically modified male mosquioes were released as part of a "*Mark-Release-Recapture*" (MRR) comparative study.

All mosquitoes were marked with a fluorescent powder to distinguish them from wild-type and facilitate identification during recapture. A molecular diagnostic was also used to identify the modified mosquitoes.

The intensive MRR study lasted for 20 days and was followed by seven months of monitoring to verify the disappearance of the transgene from the environment. The data has been reported to the ANB.

Field release results confirming data from the contained research

The outcomes for this study were consistent with expectations in observing how the insects behaved in the field, and in validating field entomological and laboratory protocols associated with mosquito rearing, marking, release, monitoring, and molecular verification. The detailed results of the release study will be published in peer-reviewed journals. The scientific goals of the release were all achieved, as summarised below:

- Non gene drive sterile male mosquitoes were recaptured in swarms, showing that they can take part in swarming activities (important for reproduction) in a similar manner to wild mosquitoes. Non-modified male mosquitoes were also recaptured as well as wild-type male mosquitoes.
- Non gene drive sterile male mosquitoes were recaptured inside homes, showing that, like wild males, they sometimes prefer to shelter inside houses.
- Non gene drive sterile male mosquitoes did not disperse beyond the release village boundaries, and were comparatively less mobile than wild type released mosquitoes.
- The survival of the released insects was also estimated. Sterile male mosquitoes did not survive as long as their released non-modified counterparts.
- Following the 20-day daily recapture period of the MRR, regular monthly monitoring assisted by molecular analyses continued for a total of 7 months and, as expected, no genetically modified mosquitoes were detected during this period.
- The release, MRR study, and monitoring activities, all proceeded as planned. All steps leading to the release, including transport and the release itself were conducted in presence of observers of the ANB who also checked all protocols. Reporting obligations have now been finalised.

From a co-development and stakeholders engagement perspective, the release showed that stakeholders have the capacity to make informed decisions based on continued dialogue. Engagement should begin early and continue across the research timeline. Bringing communities into the research pathway earlier generates a strong level of commitment to research process and findings. The stakeholder engagement team in Burkina Faso has informed stakeholders of the results and lessons learned from the release study.

Our next phase: non gene drive male bias

Building on their work with the non gene drive sterile male mosquitoes, the Target Malaria Burkina Faso team will begin their next research phase: non gene drive mosquitoes with a male bias.

The non gene drive mosquitoes carry a genetic modification that leads to fertile males which produce predominantly male offspring. When these modified males mate with wild females, the modification is passed on to half of their offspring. The strain has no fitness advantage or biased inheritance of the modification, so that if they were to be released in the field the mosquitoes would disappear over a limited number of generations.

Like the sterile males, the male bias mosquitoes will not carry the gene drive technology and are not expected to be used as an immediate tool against malaria. The male bias mosquito strain was also initially produced in the laboratory and tested in small cages at Imperial College London. It was subsequently tested in contained laboratories in large cages at Polo GGB in Italy, and will be imported from Italy to Burkina Faso provided that IRSS receives the appropriate permits and that the community surrounding the insectary gives its agreement.

Our plans for the future

The research is still in its early stages, and although initial results look promising, there is still a long way to go. We ultimately hope to develop mosquitoes that carry a gene drive element, which biases inheritance of a trait that would result in a decrease of malaria transmission by mosquitoes. Gene drive mosquitoes could, therefore, be a powerful tool for controlling malaria in Africa that would complement existing vector-control methods.

At the end of our research programme, if gene drive technology proves reliable, safe, effective and sustainable, and if it is approved by national regulatory authorities, local communities and other potentially impacted stakeholders, it will be provided for use in malaria-affected countries through an open access agreement.

Our aim is to build a forum for dialogue, trust and sharing between communities, civil society and all stakeholders in support of scientific progress. Our vision is to contribute to world free from malaria. The supportive and enabling environment the project has experienced in Burkina Faso is very encouraging at this early phase.

For now, Target Malaria has not yet planned any releases of self-sustaining gene drive mosquitoes.

1 Target Malaria Independent ecological risk assessment for a small-scale field release of a sterile male strain of *Anopheles coluzzii* *https://targetmalaria.org/wp-content/uploads/2021/02/CSIRO_Target_Malaria_Risk_Assessment_Sterile_Males_plus_Executive_Summary1.pdf*