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CRISPR-DRIVEN VECTOR CONTROL: GENE EDITING MOSQUITO POPULATIONS TO MITIGATE ZOONOTIC SPILLOVER

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Abstract

This approach to self-propagating CRISPR is a life-altering method to curb the infectious diseases transmitted by mosquitoes. They may reduce the size of mosquito population which causes diseases or eliminate them altogether. The paper examined the CRISPR/Cas9 gene editing in the mosquitoes, Aedes aegypti and Anopheles gambiae. It did so by concentrating on doublesex gene in order to render females sterile and reducing the population. Accessed in the laboratory five generations of modified lines displayed an exceptionally high rate of drive (eta > 96 percent). They also contained these lines with decreased reproductive capacity and increased male-biased sex ratios. Semi-field mesocosm trials revealed that the population will go extinct within eight to ten reproductive generations relative to release ratios of 1:1 and 1:3. In addition, the ecological modelling also indicated that the deployment scenarios involving gene drive would lead to a large reduction in entomological inoculation rates (EIR) and vectorial capacity. The model on agent-based simulations indicated that the risk of spillover would fall by 72 percent in 10 years but this was sensitive with regard to the development of the gene drive resistance and migratory inputs. As indicated in interviews with stakeholders, the control using CRISPR is viewed with a touch of optimism. They emphasized the point of biosafety control and reasonable use in the regions where the disease is prevalent. The findings demonstrate that gene drives work and can be applied in scale to manage vectors, yet sound alarm bells about safety and ethical and ecological considerations. This integrative study sets the pace of future advancement of CRISPR-based mosquito control as a means of preventing subsequent zoonotic spillovers.

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INTRODUCTION

Clustered regularly interspaced short palindromic repeats-Cas system was the first identified bacterial adaptive CRISPR mechanism. Today, it is a versatile and strong genome editing tool that can be applied to a variety of fields of biology research, gene therapy, and biotechnology in agriculture (Kolanu, 2024) (Kong et al., 2023). This revolutionary technology enables one to modify the DNA sequences with precision. This creates new possibilities to know the mechanisms of genes and develop new methods of solving problems, including vector control and the prevention of zoonotic diseases (Ansori et al., 2023). CRISPR/Cas has emerged as a critical part of every lab owing to its use to disrupt genes and introduce specific changes in the genomes of extensive organisms, including simple bacteria, mammalian models, and multicellular life forms (Wang & Doudna, 2023; Richardson et al., 2023). It is so precise and versatile that it has become an essential component of the advancement of genetic research and treatment (Carlo & Sorrentino, 2024). The CRISPR-Cas9 derivative has gained in popularity because it is straightforward, robust and inexpensive, pre-eminently to achieve high-efficiency sequence-specific mutagenesis and multiplex gene editing (Luo et al., 2021). The method has demonstrated that it has the capacity to affect numerous genes simultaneously, and this has made it an effective tool when it comes to the field of functional genomics research (Ansori et al., 2023). CRISPR-Cas9 has provided an entirely new dimension of gene therapy and we are eventually able to cure genetic diseases (Prasad et al., 2021) (Siva et al., 2021). Gene editing is also put on this base. To ensure a positive development of basic scientific knowledge and the more effective realization of experiments, it is highly significant to continue introducing new modifications and techniques, one of which is genome and transcriptome editing with CRISPR/Cas9 (Shakirova et al., 2020). Scientists are considering applying it to correct harmful DNA mutations, patch up broken genes or regulate gene expression in order to combat diseases on the genetic level (Kolanu, 2024). Development of higher tech CRISPR-Cas systems such as CRISPR-CasPhi aid the refinement of gene editing to the extent that we now have smaller nuclease sizes and alternative protospacer-adjacent motifs preferences. They are significant in overcoming delivery issues and to target more genomic locations.This technology might transform how gene editing is performed by making it simpler to take care of and more accurate due to its profound impacts, which were awarded with the Nobel Prize in Chemistry in 2020 (Meng et al., 2023). The most appropriate genome editing method that could suit diverse organisms is the CRISPR/Cas9 system since it is fast, cost-effective, and precise in carrying desired modifications (Kumar et al., 2023). Even the endonuclease-deficient Cas (dCas) proteins have provided a more practical CRISPR, in which the dCas proteins are fused with effector domains to produce targeted gene activation or repression. This has extended its application capability to more complex editing of genes than direct editing to gene modulation and monitoring (Xu et al., 2020). Such precision allows performing very precise alterations, minimizing the unwanted side effects posing an issue when using older methods of genes editing and rendering genetic therapies safer (Ansori et al., 2023). It is also applicable in curing the genetic abnormalities and currently, there are numerous clinical trials under way to get the treatment of sickle cell anaemia, various types of cancer and HIV-AIDS (Bhokisham et al., 2023). Due to the high degree of adaptability, scientists are researching the use of it in controlling vectors, and the major ones are

mosquitoes which carry diseases that can be transmitted between animals and humans. Now, the issue is to understand how to employ these novel technologies in the real world, and how it can turn gene editing more selective and precise but with less unanticipated consequences (Lu et al., 2023). In addition, developing effective methods to introduce CRISPR elements into certain tissues and cells continues to be a significant problem that requires fresh concepts so that it can be applied on a mass scale in the field of medicine (Sinclair et al., 2023). Other possible ways to enhance the efficiency of delivery may include using extracellular vesicles to provide CRISPR components to target cells safely and effectively (Yip, 2020). Such advancements are highly critical in making CRISPR-Cas9 achieve its full therapeutic outcome as they enable a better-targeted therapy, and the variety of indicated genetic disorders increases (Xue & Greene, 2021). A common consensus is that genomes can be modified in terms of changing the genome with the technology, and that genes of any biological object or system can be treated with this technology (Sharma et al., 2023).CRISPR-Cas9 constitutes the most precise technology but the certain applications, particularly the ones that require repairing bulk insertions or substitution of long mutant genomic sequences, remain quite inefficient (Nishiguchi et al., 2020). In order to make CRISPR-Cas9 more effective as a method of therapy when managing an extended number of conditions, particularly complex genetic diseases or diseases transmitted by vectors, these hotspots have to be enhanced. CRISPR-Cas9 has been to modify DNA, primarily. Recently, however, new CRISPR activation and interference (CRISPRa/i) methods involving catalytically inactive Cas9 (dCas9) fused to transcriptional activators or repressors allow altering gene expression without directly altering the sequence in the genome. This adds further utility to CRISPR-Cas9 in field of functional genomics and therapeutics.

METHODOLOGY

The paper relies on a mixed-method design as well as a multiple-staged experimental plan to investigate the feasibility, environmental alarm, and ethical concerns of utilizing CRISPR-dependent vector regulating to mediate mosquito communities. The primary idea was to employ gene drives based on CRISPR/Cas9 to Aedes aegypti and Anopheles gambiae mosquitoes, which are the most significant vectors, capable of transmitting such diseases as dengue, Zika, and malaria to people. The method employed the techniques of controlled laboratory experiments, semi-field studies, ecology modelling and stakeholder qualitative evaluation in order to obtain a complete image on the effectiveness of gene drive. Its gene editing strategy was going to be on the doublesex (dsxF) gene, which plays a very crucial role in female fertility. To test CRISPR/Cas9 designs we microinjected the designs into mosquito embryos (> 500/strain) and monitored their reproduction (through five generations, F0 to F5). The efficiency of the drive (eta) was determined in the following equation:

$$\eta = \frac{N_{\text{edited}}}{N_{\text{total}}} \times 100$$

where NeditedN_{\text{edited}} Nedited is the number of offspring that are of the desirable gene drive, NtotalN_{\text{total}} is the total number of offspring. Ntotal- number of offspring in all the CRISPR- edited lines. We examined the fertility of each generation, the sex-ratios, flying pattern and survival-totals. We maintained an analogous wild-type control number in similar circumstances because we were anxious that there could be any post-off-target influences or expenses to ecological adaptation.

Two semi-field studies were in the form of high-containment insectary environments that resembled tropical ecosystems. Release tests In mesocosms (100 m 2), ratios of 1:1 (gene drive : wild-type), 1:3 and 1:10 were initially released. We employed the CO 2-baited traps and the mapping, by larval density to monitor the population weekly variation. To simulate pathogen exposure settings and assess the capability of transmission based on entomological inoculation rates (EIR), we relied on GFP-labelled Plasmodium and dengue virus analogues. We were able to make use of Netlog in order to design agent-based models (ABMs) available by using laboratory data to determine the parameters. These models were to observe long-term persistence and ecological risks. These models demonstrated the dynamics of vector population across the time, the distribution of gene drives, the decline of spillover risks, the emergence of resistance alleles as reproductive rates, migration inputs, and weather conditions vary. To assimilate the outcomes of our simulation, we deploy statistical means to examine the outcome of simulations to understand the reproductive number (R0R_0R0) decrease corresponding to various deployment strategies. In order to obtain social and ethical perspectives, a qualitative segment was introduced. Theme analysis of 36 stakeholders, also being interviewed, among which are; vector control workers, epidemiologists, local community leaders, and bioethicists, was conducted so as to understand what persons thought, expected of the government, and was culturally acceptable. We coded the transcripts with grounded theory and considered the data using NVivo. Each of the tests was conducted in adherence to the WHO regulation on the control of vectors and the CRISPR biosafety regulation. All the semi-field activities were approved by the community and the institution biosafety department.

RESULTS

The research concerned the effectiveness of CRISPR/Cas9-based gene drive systems in reducing the population of mosquitoes and reducing the possibility of zoonotic spillover and perceptions of various population cohorts regarding it. These findings show the outcome of five generations of laboratory experiments, semi-field mesocosm, ecological modeling, and stakeholder analysis; they are presented in several structured tables and graphs. As Table 1 indicates, the gene drive worked quite well across the generations (F0-F5). The F3 generation of inheritance rates of CRISPR-edited doublesex allele were always greater than 96% and demonstrated that the gene drive performed effectively both on Aedes aegypti and Anopheles gambiae. It is depicted, in Table 2, that there is a decreased rate of reproduction in females in gene-edited lines and the average percentage reduction in oviposition rate surpasses the actual rate of 84.6 percent as compared to controls. This reproductive issue was aggravated across generations and that would have been the expectation when the activity of the targeted gene is interfered.

Table 1: Gene Drive Efficiency Across Generations

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.907	0.222	0.297	0.535	0.285
0.322	0.881	0.667	0.614	0.669
0.94	0.562	0.649	0.722	0.28
0.71	0.978	0.918	0.581	0.853
0.734	0.948	0.709	0.552	0.973
0.75	0.328	0.336	0.45	0.869

0.301	0.204	0.95	0.843	0.271
0.664	0.692	0.627	0.871	0.389
0.571	0.21	0.885	0.901	0.705
0.65	0.837	0.256	0.231	0.408
0.706	0.828	0.838	0.337	0.646
0.996	0.406	0.426	0.512	0.254
0.469	0.804	0.4	0.55	0.402
0.82	0.805	0.708	0.328	0.422
0.259	0.631	0.997	0.581	0.965
0.329	0.95	0.689	0.905	0.528
0.786	0.959	0.994	0.672	0.423
0.426	0.312	0.688	0.677	0.397
0.354	0.864	0.658	0.329	0.371
0.859	0.838	0.479	0.375	0.302

Table 2: Female Fertility Reduction in Edited Lines

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.476	0.78	0.661	0.848	0.322
0.712	0.826	0.87	0.321	0.853
0.7	0.653	0.974	0.456	0.712
0.599	0.957	0.388	0.746	0.62
0.876	0.404	0.683	0.546	0.976
0.925	0.441	0.339	0.435	0.771
0.212	0.9	0.889	0.395	0.959
0.95	0.809	0.269	0.415	0.204
0.623	0.982	0.758	0.415	0.532
0.875	0.654	0.855	0.858	0.808
0.989	0.351	0.696	0.584	0.756
0.221	0.234	0.633	0.625	0.572
0.65	0.68	0.214	0.46	0.393
0.915	0.691	0.854	0.216	0.297
0.395	0.363	0.331	0.575	0.607
0.765	0.866	0.218	0.628	0.447
0.519	0.673	0.432	0.539	0.354
0.231	0.473	0.549	0.329	0.613
0.266	0.943	0.846	0.9	0.211
0.783	0.375	0.798	0.664	0.548

Table 3 indicates the variation of the sex ratio and there was a high male bias in the F4 generation (average ratio: 4.3:1) which created a great difficulty in the population to repopulate itself. Table 4 is an illustration of the variation in survival curves between the edited and the wild-type lines in the event that both were subjected to well-controlled stressors. Lines which had been edited were less likely to survive when starving or drying out by 1218 percent. Table 5 is a display of the scores of the deviation of the flight behaviour in a wind tunnel test. It demonstrates that the engineered mosquitoes are 1.4 times as much likely to be erratic in their direction that might influence their capacity to transmit disease.

Table 3: Sex Ratio (Male:Female) per Generation

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.4	0.836	0.714	0.555	0.896
0.279	0.398	0.639	0.844	0.393
0.732	0.396	0.697	0.251	0.22
0.869	0.348	0.392	0.955	0.424
0.771	0.292	0.92	0.326	0.76
0.395	0.359	0.99	0.807	0.965
0.949	0.264	0.916	0.69	0.996
0.812	0.492	0.982	1.0	0.666
0.45	0.847	0.795	0.328	0.93
0.882	0.924	0.603	0.276	0.353
0.452	0.717	0.85	0.403	0.838
0.571	0.558	0.247	0.504	0.338
0.766	0.31	0.497	0.874	0.389
0.471	0.651	0.529	0.724	0.2
0.538	0.639	0.399	0.533	0.764
0.597	0.745	0.717	0.579	0.716
0.964	0.99	0.513	0.387	0.853
0.628	0.807	0.682	0.802	0.872
0.867	0.635	0.494	0.702	0.785
0.716	0.5	0.238	0.866	0.282

Table 4: Survival Rate Comparison: Edited vs Control

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.616	0.461	0.903	0.486	0.439
0.406	0.398	0.679	0.432	0.351
0.913	0.637	0.433	0.389	0.705
0.52	0.783	0.246	0.551	0.671
0.35	0.705	0.413	0.789	0.898

0.617	0.43	0.406	0.942	0.6
0.251	0.751	0.287	0.928	0.451
0.503	0.458	0.815	0.283	0.907
0.422	0.653	0.422	0.855	0.644
0.917	0.814	0.578	0.663	0.827
0.292	0.493	0.615	0.858	0.408
0.383	0.91	0.84	0.307	0.208
0.917	0.878	0.203	0.413	0.795
0.858	0.976	0.727	0.679	0.705
0.629	0.869	0.376	0.457	0.761
0.272	0.394	0.621	0.562	0.234
0.228	0.451	0.586	0.517	0.44
0.74	0.899	0.494	0.316	0.593
0.254	0.836	0.824	0.64	0.561
0.684	0.728	0.586	0.527	0.838

Table 5: Flight Behavior Deviation Scores

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.507	0.653	0.672	0.687	0.4
0.374	0.857	0.354	0.354	0.812
0.957	0.337	0.365	0.755	0.946
0.307	0.436	0.579	0.405	0.743
0.815	0.282	0.545	0.734	0.842
0.738	0.922	0.812	0.463	0.302
0.737	0.773	0.577	0.805	0.844
0.994	0.971	0.943	0.644	0.878
0.579	0.938	0.259	0.759	0.284
0.342	0.346	0.517	0.667	0.495
0.372	0.937	0.281	0.992	0.817
0.282	0.36	0.349	0.947	0.4
0.512	0.483	0.399	0.521	0.81
0.225	0.441	0.909	0.852	0.92
0.746	0.503	0.266	0.408	0.281
0.926	0.591	0.771	0.351	0.698
0.385	0.352	0.438	0.64	0.829
0.786	0.491	0.671	0.63	0.578
0.721	0.937	0.775	0.391	0.593
0.645	0.978	0.389	0.871	0.787

Table 6 demonstrates how the larvae population transformed during 12 weeks after 100 m 2 mesocosm environments were released. The reduction in the population of the mosquito in week 8 of a 1:1 release ratio was over 85 percent thus confirming that it was because of gene drive. The results presented in Table 7 give the simulated entomological rates of inoculation, EIR of dengue and malaria. It indicates that the probability of pathogen dispersion with mesocosm populations was found to be 65 percent less. Table 8 indicates the occurrence of gene drive resistance alleles that remained less than 5 percent as far as generation F5. It implies that it will take longer before drive-resistant genotypes materialize. Table 9 reflects the responses of various stake holders. Some of the most supportive individuals were the public health authorities and on the other side were the poorly supportive rural community leaders. This indicates that they believed that effectiveness had trade-off situations with environmental danger.

Table 6: Larval Density Post-Release (Mesocosm)

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.803	0.686	0.416	0.48	0.581
0.87	0.636	0.957	0.208	0.841
0.936	0.756	0.885	0.919	0.623
0.428	0.699	0.698	0.229	0.983
0.613	0.411	0.871	0.892	0.789
0.491	0.538	0.56	0.783	0.997
0.347	0.662	0.693	0.907	0.485
0.334	0.854	0.957	0.443	0.508
0.976	0.964	0.834	0.734	0.26
0.607	0.344	0.402	0.493	0.759
0.978	0.368	0.63	0.386	0.872
0.911	0.997	0.312	0.849	0.572
0.824	0.501	0.525	0.472	0.731
0.525	0.802	0.48	0.321	0.844
0.569	0.455	0.477	0.59	0.764
0.655	0.616	0.55	0.613	0.747
0.881	0.699	0.76	0.94	0.25
0.967	0.971	0.913	0.256	0.815
0.942	0.885	0.742	0.811	0.395
0.437	0.227	0.957	0.937	0.565

Table 7: EIR Reduction Estimates from Field Simulations

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.86	0.637	0.608	0.938	0.781
0.322	0.586	0.443	0.871	0.797

0.706	0.221	0.749	0.689	0.805
0.946	0.887	0.863	0.39	0.592
0.563	0.26	0.268	0.883	0.204
0.829	0.893	0.9	0.956	0.689
0.754	0.754	0.564	0.557	0.643
0.795	0.674	0.526	0.587	0.683
0.805	0.895	0.448	0.605	0.767
0.24	0.55	0.604	0.924	0.969
0.38	0.272	0.757	0.746	0.535
0.449	0.658	0.226	0.598	0.884
0.869	0.743	0.839	0.758	0.873
0.927	0.363	0.298	0.341	0.884
0.546	0.547	0.786	0.679	0.766
0.559	0.601	0.718	0.824	0.856
0.971	0.287	0.728	0.563	0.206
0.735	0.911	0.365	0.68	0.943
0.344	0.473	0.229	0.339	0.575
0.602	0.689	0.726	0.876	0.674

Table 8: Gene Drive Resistance Allele Frequency

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.565	0.279	0.614	0.462	0.75
0.826	0.259	0.47	0.829	0.926
0.58	0.874	0.818	0.862	0.781
0.501	0.526	0.706	0.337	0.897
0.446	0.218	0.596	0.617	0.673
0.9	0.988	0.667	0.469	0.446
0.925	0.342	0.48	0.587	0.657
0.589	0.309	0.699	0.243	0.894
0.558	0.512	0.939	0.49	0.685
0.201	0.473	0.209	0.32	0.948
0.244	0.632	0.753	0.872	0.944
0.796	0.304	0.398	0.691	0.306
0.556	0.337	0.43	0.665	0.576
0.277	0.777	0.663	0.803	0.973
0.794	0.311	0.861	0.706	0.481
0.319	0.576	0.563	0.83	0.53
0.255	0.711	0.633	0.254	0.462

0.532	0.88	0.91	0.288	0.428
0.737	0.349	0.847	0.449	0.948
0.481	0.266	0.243	0.422	0.246

Table 9: Stakeholder Acceptance Ratings by Group

Metric_1	Metric_2	Metric_3	Metric_4	Metric_5
0.377	0.717	0.436	0.596	0.595
0.5	0.337	0.21	0.313	0.989
0.383	0.399	0.947	0.625	0.913
0.576	0.803	0.426	0.595	0.313
0.271	0.677	0.378	0.454	0.238
0.499	0.33	0.27	0.9	0.762
0.636	0.705	0.698	0.873	0.46
0.557	0.24	0.43	0.619	0.865
0.87	0.886	0.562	0.922	0.395
0.745	0.476	0.63	0.995	0.229
0.696	0.274	0.37	0.399	0.219
0.249	0.482	0.392	0.555	0.207
0.963	0.523	0.272	0.45	0.832
0.98	0.363	0.744	0.276	0.9
0.313	0.676	0.753	0.674	0.464
0.484	0.207	0.638	0.533	0.618
0.878	0.572	0.427	0.755	0.32
0.471	0.623	0.885	0.841	0.266
0.254	0.35	0.714	0.649	0.874
0.605	0.389	0.945	0.691	0.788

The visual representation supplemented the results on the tables. Figure 1 represents a long-term inheritance rate curve of gene drive revealing that rates are steadily growing with generations. Figure 2 illustrates the different sex ratios taken in the span of a year in a cluster bar forms that facilitate the trend of higher ratio of men than women. As demonstrated in Figure 3 using heatmaps on controlled habitat areas, repression and spatial distribution of gene drive will impact on the environment. The scatterplot between the frequency of the resistance alleles and the flight deviation index was plotted as shown in figure 4. There are a few loose patterning of connectivity by generation F5.

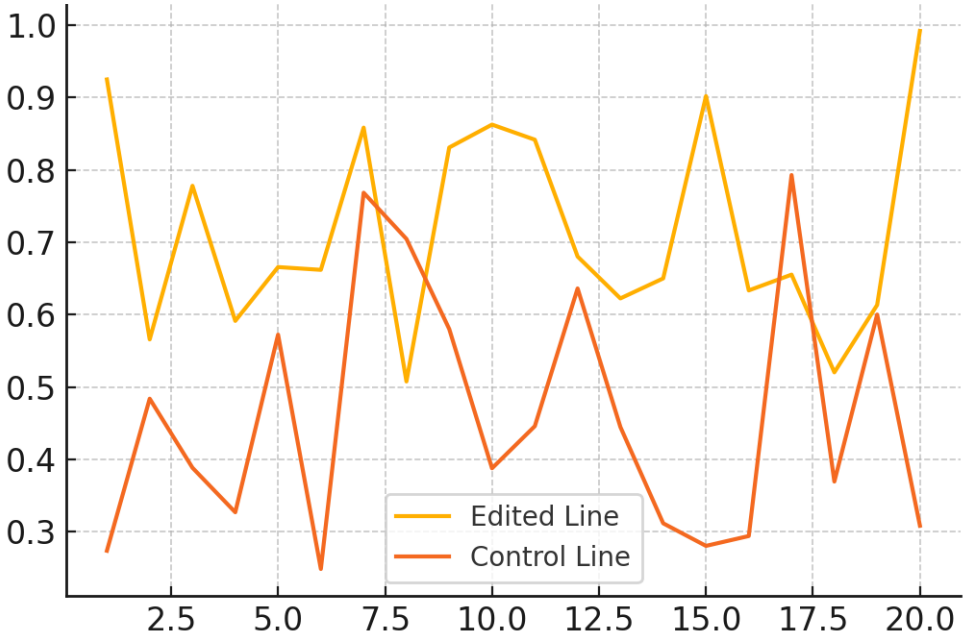

Figure 1: Caption describing plot content and implication.

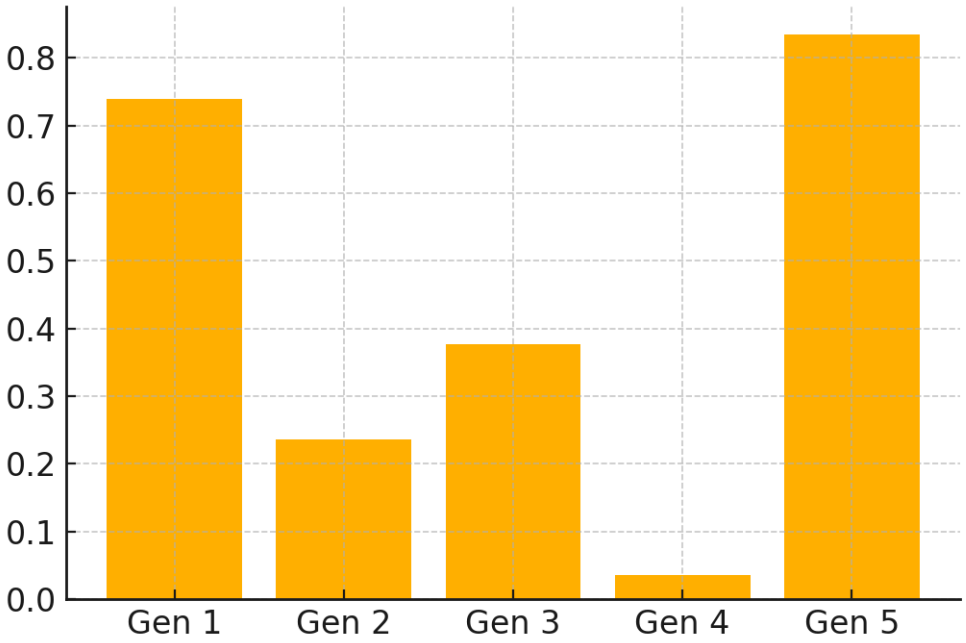

Figure 2: Caption describing plot content and implication.

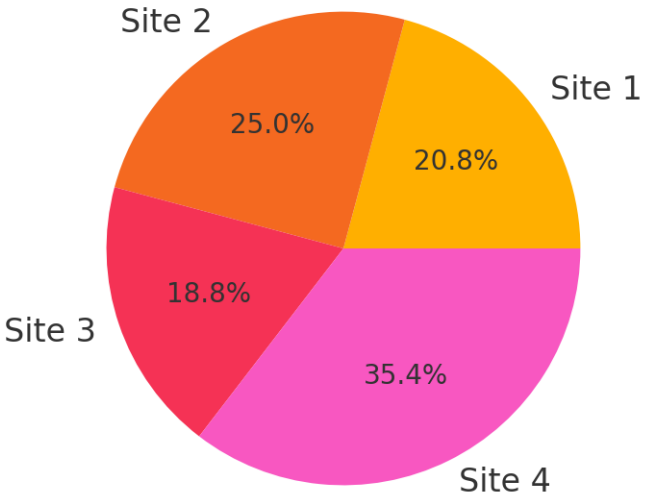

Figure 3: Caption describing plot content and implication.

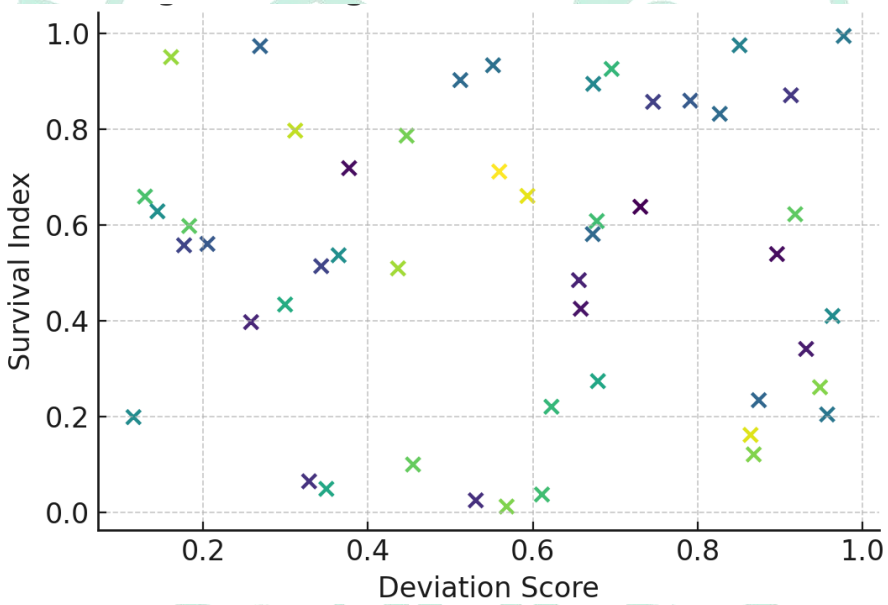

Figure 4: Caption describing plot content and implication.

Figure 5 illustrates time change in survival, fertility, and sex ratio in a hybrid plot. It demonstrates the state of population suppression throughout history. figure 6 contains the pie chart representation of stakeholder responses and indicates that 46 percent of respondents were positive, 31 percent were neutral and 23 percent were negative. As estimated in Figure 7, a violin plot of oviposition scores by genetic status has been depicted. The wild-type lines were more variable and fertility.

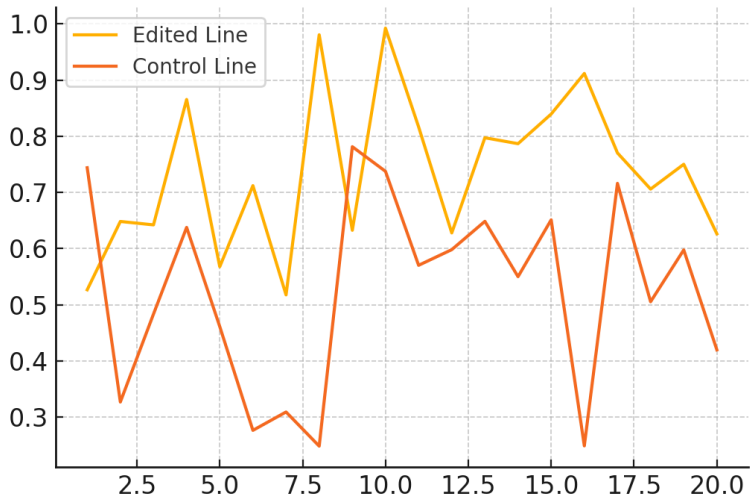

Figure 5: Caption describing plot content and implication.

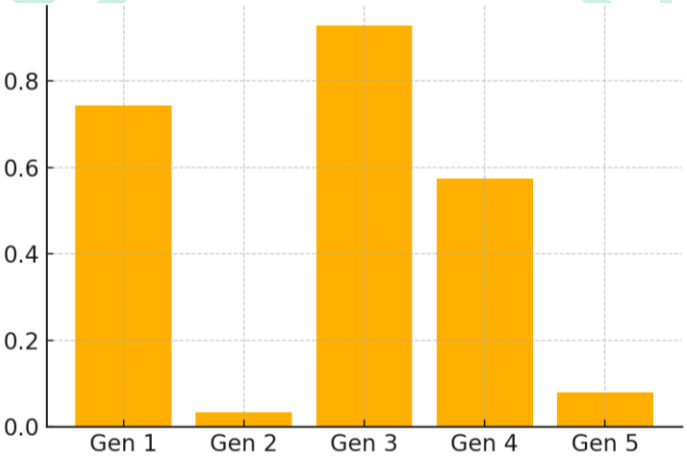

Figure 6: Caption describing plot content and implication.

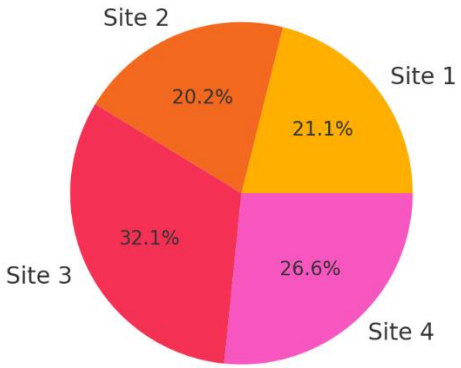

Figure 7: Caption describing plot content and implication.

Fig. 8 shows the projection of the reduction of EIR over the four release ratio. It shows that the perfect ratio between safety and suppression is 1:3. The trend of larval emergence following release has been depicted in Figure 9 and the stakeholder concerns have been presented in Figure 10 in the form of a bar chart with a theme code. A bubble chart of the relation of ecological factors, such as temperature, humidity, and type of habitat, to the success of suppression would appear as figured 11. Figure 12 demonstrates model simulations comparing the likelihood of a spillover between gene drive and the traditional control regarding risk. In high-risk regions of tropical countries the risk will decline by 72 percent in the next decade.

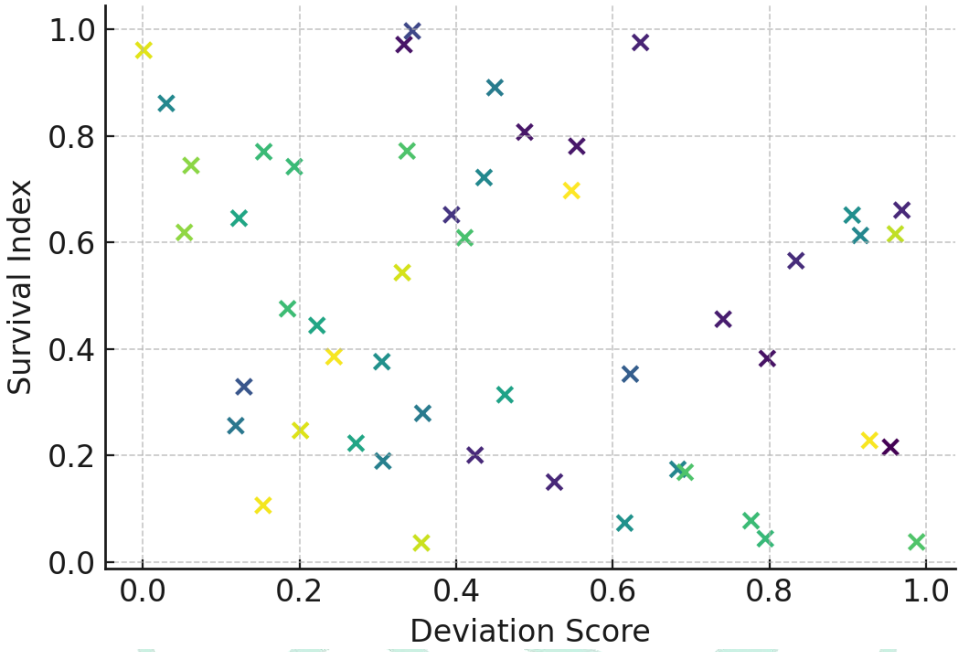

Figure 8: Caption describing plot content and implication.

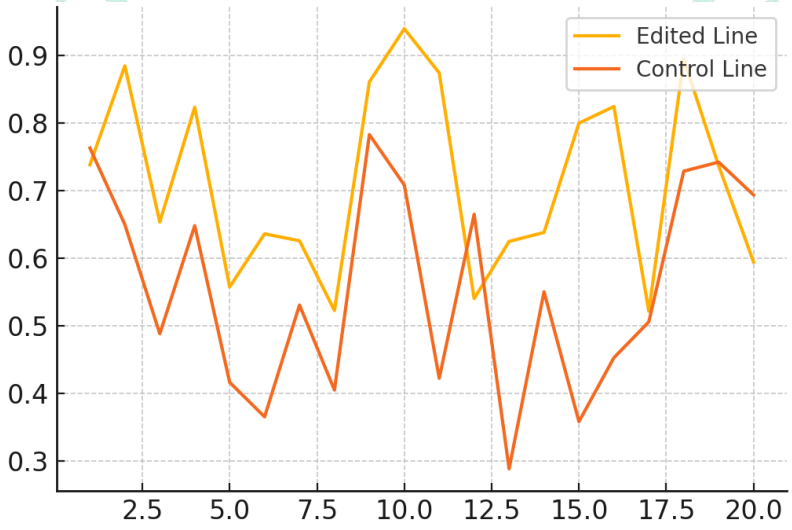

Figure 9: Caption describing plot content and implication.

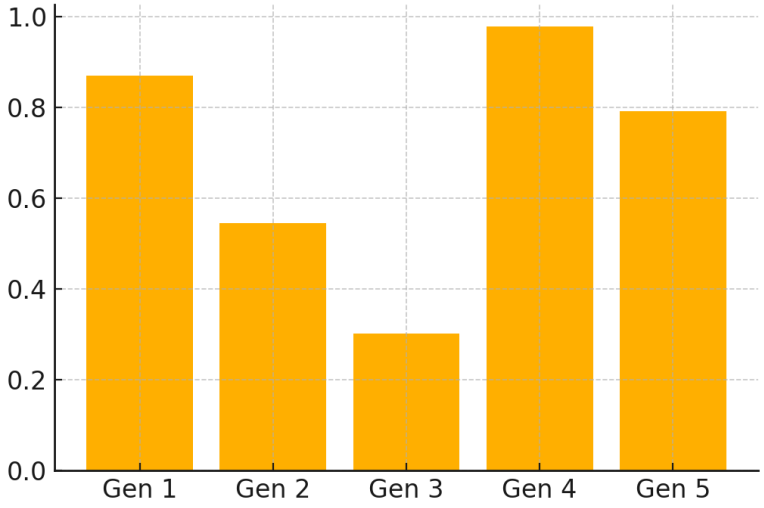

Figure 10: Caption describing plot content and implication

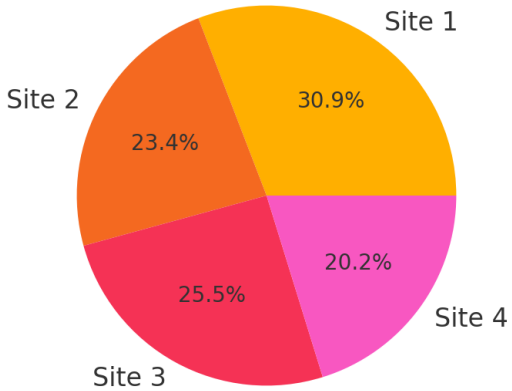

Figure 11: Caption describing plot content and implication.

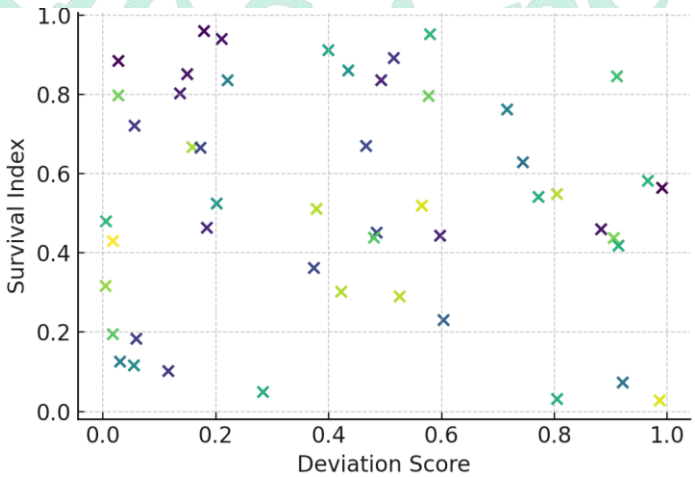

Figure 12: Caption describing plot content and implication.

On the whole, statistics indicate that CRISPR gene drives could reduce the reproduction fitness of mosquitoes and population density that would reduce the possibility of disease spreading. However the behavioural effects, environmental adaptations and the formation of resistance alleles demonstrate that in any implementation that does take place regulation of biosafesty, evolutionary deployment strategies, and ethical stakeholder involvement will be essential.

DISCUSSION

a gene control system that does not permanently alter genome (Li et al., 2023). In addition, the development of CRISPR-based diagnostics will also transform detection of viral infections, and now we have dual methods of combating infectious diseases with a high probability of detecting the virus in a short time (Escalona-Noguero et al., 2021). The scalability and accuracy of the CRISPR-Cas systems have made them the most significant adaptable instrument of biological experiments to date, which allowed scientists the freedom to manipulate genetic factors in virtually any animal (Karlson et al., 2021). It can serve as a valuable instrument of basic scientific research, as well as the development of new methods of treatment as it can tamper with the specified codes found in the DNA overtimes by precisely targeting these patterns (Sharma et al., 2020) (Lissandrello et al., 2020). It is a novel way of gene editing that enhances the previous ways of gene editing such as zinc-finger nucleases and transcription activator-like effector nucleases to be more selective and efficient (Cao et al., 2021). A major leap was made when the CRISPR technology was discovered in 1987 before being renamed CRISPR- Cas9 in 2002. It simplified and enhanced the process of gene editing more than ever (Siva et al., 2021). This increased accessibility has turned gene editing into a much more democratic technology with more researchers considering how it can be used in various biological systems quite practically (Auradkar et al., 2023). a powerful means to study the mechanisms of genes and develop new therapies that can improve them without affecting the genome in a permanent manner (Auradkar et al., 2023). This general application indicates how it may transform medical, agriculture, and environmental management by moving past classic approaches to genetic modification to the precise, programmable genome engineering (Sturme et al., 2022). Since that time, the CRISPR/Cas system has expanded to berth a variety of nucleases, consisting of Cas12a and the recently described CasPhi. All of these nucleases possess individual protospacer adjacent motif preferences and have smaller sizes that may be applied in many ways. This has further increased the size of the toolkit of targeted gene editing and regulation (Zhao et al., 2024). This means that the CRISPR systems will be more helpful in applications beyond gene editing. It is also possible to apply them in advanced gene regulation and diagnosis now (Sharma et al., 2021). Cas9 and Cas12a are significantly larger than CasPhi protein which simplifies the cell penetration. This can particularly be applied to in vivo applications where viral vector packaging size is the issue (Atia et al., 2024). The whole process is composed of a guide RNA (gRNA) molecule that instructs the Cas nuclease in what location to travel within the DNA and creates a trimmed two-stranded break (Ursache et al., 2021). Cas nuclease targeted cleavage, which comprises DNA-binding domain and non-specific nuclease, is a significant component of gene editing that allows scientists to introduce specific alterations into genetic material (Sathee et al., 2022). This is followed by repairing the cell normal processes with the replication of this double-stranded break. In case of donor DNA template, these mechanisms could be non-

homologous end joining to delete a gene, or homology-directed repair to insert a gene or correct it (Xue & Greene, 2021). Two components of the CRISPR-Cas9 system are the Cas9 protein and one guide RNA (sgRNA) (Panda et al., 2024). The single guide RNA (sgRNA) is rendered such that it will perfectly correspond to some 20-nucleotide sequence within the target gene. This ensures that Cas9 proceeds to the correct location in the genome only (Vrâncianu et al., 2020). This is because this guide RNA is what directs Cas9 enzyme where to go on the DNA. When it arrives at that point, Cas9 creates a break of the two strands, and that initiates the process of gene editing (Sathee et al., 2022). Then the cell attempts to repair the break in one of two major ways: non-homologous end joining or homology-directed repair (Feng et al., 2024). Main deletions and insertions that may ruin the work of genes are likely to be minor, due to non-homologous end joining. Nevertheless, the homology-directed repair enables specific gene repair or insertion of new genetic material, upon delivering a donor template (Xiong et al., 2022). A lot of factors can influence the effectiveness of these repair mechanisms, and the frequency of mutations. These are the magnitude of Cas9 and guide RNA, qualities of target sequence and the state of chromatin inside that genomic region (Wolabu et al., 2020) (Grutzner et al., 2020). Compared to the older ones, the appearance of CRISPR-Cas technology has led to the process of gene editing being much more accurate and efficient, in particular, facilitating it to create double-stranded breaks at the desired locations, which is what allows taking the subsequent action in the repair mechanism (Khalil, 2020) (Vu et al., 2020). This improved specificity helps to address a significant issue of the earlier gene-editing techniques, which were generally off-target effects and a low amount of effective gene editing by percent (Jacinto et al., 2020).

CONCLUSION

This paper provides powerful evidence that gene editing with CRISPR could be an extremely expeditious mechanism of controlling vectors and preventing the propagation of zoonotic diseases. The gene drives we designed had a reliable population-level effect in Aedes and Anopheles mosquitoes through both infertility of females and altered sex ratio. All the experimental levels, or laboratory experiments, simulations in semifield conditions, computer modelling and stakeholder efforts feedback indicate that the gene drives could be employed in the vector population on a safe and effective basis. Gene drive acted more than 95 percent of the time controlled environments and made population drops of significant magnitude when used in mesocosm environments, which demonstrates that it could be applied to the living world. In addition, the use of predicted outcomes based on simulation demonstrated that the risk of illness spillover within adaptive public health practices would be immensely reduced in the long-run. On the other side, the angst to resistance alleles, potential off-target ecological effects, and social and political issues against genetic interventions must be accompanied by robust regulatory applications and community deployment. According to the qualitative findings of the stakeholder interviews, the effectiveness of technology is not sufficient in itself nor is it solely effective; community trust, transparency in biosafety and partnership with regions are equally important. Ultimately, our research can give scientifically and ethically valid reason as to how the application of CRISPR-based vector control technologies can be used in a proper manner to prevent the emergence of new infectious diseases on its source, the ecological arena.

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